AgNOR SCORE IN CERVICAL LESIONS

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CERTIFICATE

This is to certify that the dissertation entitled ***"AgNOR SCORE IN CERVICAL LESIONS"*** *submitted by* ***Dr. S. Shanmugavalli*** *to the Faculty of Obstetrics and Gynaecology, The Tamilnadu Dr. M.G.R. Medical university, Chennai in partial fulfillment of the requirement for the award of M.D. Degree Branch II (Obstetrics and Gynaecology) is a bonafide research work carried out by her during the period of May 2005 to May 2006 under our direct supervision and guidance.*

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CONTENTS

INTRODUCTION

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Carcinoma of the cervix is the commonest type of Malignancy of female genital tract in India (Devi and Prabhavathy (1961).

By virtue of its accessibility carcinoma of the cervix can be readily diagnosed even in it's preinvasive stage. If it is treated in early stages, the patient can be cured of the disease.

For each cancer of the body of the uterus, there are 25 cases of cancer of cervix in India (Roy Chowdhury, 1975).

A close quarter observation on the social behaviour of our society reveals that more of the women have their marriage at very early part of their life leading to early age at first intercourse and poor sexual hygiene which are considered to be important etiological factors for the carcinoma cervix.

The misery of woman due to cervical cancer, which is the commonest cancer in a developing country like ours is a scourge of humanity. Early detection and management of squamous intra epithelial lesions (SIL) is the best approach to achieve control over cancer cervix. Cytological screening by pap smear has brought down the incidence of cervical cancer in developed countries. Better information can be provided by a molecular tumour marker. One such molecular tumour marker is AgNOR, which stands for silver stained (Ag) nucleolar organizer regions (NORs).

Nucleolar organizer regions (NORs) are loop of ribosomal DNA located in the short arms of acrocentric chromosomes 13,14,15, 21 and 22 and transcribe to ribosomal RNA. NORs vary in size and shape according to nucleolar transcription. They are intimately related to cell cycle and may be related to proliferation and ploidy.

AgNORs are argyrophilic proteins. Binding of silver and protein occurs in carboxyl and sulphydryl groups by colloidal precipitation of ionic silver. The carboxyl groups on the protein reduces the silver solution forming micro nuclei of silver. The large aggregates of silver are deposited at disulphide and sulphydryl group sites. These are seen by light microscopy as black intranuclear granules.

AgNOR count is an important index for assessment of proliferation of cells. In normal cells, the AgNORs are tightly packed in the nucleoli and are discernible. In rapidly proliferating cells such as neoplastic cells, nucleolar disaggregation may take place resulting in dispersion of individual AgNOR. Recent studies show that AgNOR are significantly more in malignant cells than in normal cells.

In a normal cell, 20 black dots of AgNORs should be seen (2 per arm of chromosome i.e. 2 x 10 = 20) but only one or two dots are seen as the dots are tightly packed. As we move from normal cells towards the dysplastic cells and malignant cells, the amount of DNA increases, and the number of AgNOR dots (AgNOR Count) also increases. It reflects current phase of transcription.

The AgNOR technique provides an index of cell proliferation. The number, shape and distribution of AgNOR dots counted in the cell gives information not only about the morphology but also about the behaviour of the cell. It is useful in differentiating doubtful cases of CIN.

AgNOR counts also has prognostic significance. CIN lesions with low AgNOR counts are more likely to regress in comparison to CIN lesions with high AgNOR counts. It is a simple, efficient and inexpensive method, which can be used as an adjunct to routine cytology and histopathology for diagnosis of cervical lesions especially in doubtful cases.

In the present study AgNOR counts of cervical lesions is compared with those of HPE. The results are analysed for statistically significant difference in different groups.

AIM OF THE STUDY

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1. To screen women with abnormal symptoms by speculum examination.

2. To take punch biopsy of abnormal cervix for histopathological analysis with H&E stain and AgNOR scoring with silver stain.

3. To correlate AgNOR counts of cervical lesions with histopathology.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Cell kinetics plays an important role in tumour behaviour. Proliferation rates of the tumour can be assessed to determine the behaviour of a particular tumour. The cell cycle can be divided into four phases based on the nuclear chromatin activity (Diag – 1). They are S, G_1, G_2 and G_0 phases. The cells show active reduplication of DNA in the S phase. There is a short resting phase of the cell undergoing replication at the 'S' phase. G_2 is the second resting phase before active mitosis. Thus, the DNA content at the end of 'S' phase is an indicator of proliferative activity. AgNOR detects the DNA content at this stage.

Stable cells are quiescent cells in G_0 phase and can be stimulated into G_1 by an appropriate stimulus. The tumour suppressor gene P53 blocks the progression of cells through the cell cycle late in the G_1 phase of replication. Mutant forms do not have this effect.

During the phase of active DNA replication, strips of DNA containing RNA genes are seen inside the nucleolus. These DNA fragments are actively transcribing with the help of polymerase I enzyme. These are considered as ribosomal factories. Cell cycle is controlled by a few enzymes called M phase promoting factors.

This factor has two subunit proteins.

1. 34 Kd protein, which is the product of the Cdc2 gene.
2. 45 Kd subunit consisting of proteins, which accumulate during cell cycle and are destroyed by the end of mitosis.

These proteins are named as "cyclins". The inactivation of these cyclins, inactivates M phase promoting factors also and so, this protein is required to end mitosis in a cell cycle.

Other important molecular events i.e. phosphorylation and dephosphorylation are needed to control various cellular events in mitosis and interphase. Many substrates have been identified for the P_{34} kinase and other cyclins, histones (in nuclear envelope breakdown), PP_{60} and nucleolin. Nucleolin is a major argyrophilic protein controlling the entry of cells into mitosis. Other nuclear proteins incorporated in the argyrophilic reaction are RNA polymerase I enzyme (important in transcription) no. $28/B_{23}$ protein as found by Jordan G_{47} (1987).

According to Belerguer P et.al (1996) nucleolin plays an important role in DNA transcription. It is responsible for the entry of cells into mitosis. It is the major substrate for the 34cdc2 kinase system, which controls the cell entry into the premitotic and mitotic phase. It controls the RNA splicing through its action on rDNA and the formation and assembly of ribosomes. P34 cdc2 phosphorylates the nucleolin and thus causes major conformational changes in the DNA and this results in transcription and hence the argyrophilia of nucleolin as found by Peter M (1990).

Underwood and Giri (1988) found that No.28/B23 functions in the intranuclear assembly of periribosomal particles. Thus these NORs visualize the ribosomal gene activity and hence functional protein demand of the cell. This increased protein demand and thus the proliferative rate of these cells is indicated by silver staining. NORs exist in constant positions on short arm

of acrocentric chromosomes namely 13,14,15,21 and 22. These are clearly demonstrated at metaphase. Twenty NORS could be demonstrated at this phase. This is because NORs occur in pairs on the acrocentric chromosomes.

The precise localization of NOR, has been found to be in the 18S rDNA gene cluster in the stalk region. However, exceptions do occur.

An increase in the number of AgNORs counted in any given section could be explained as follows.

a. Increase in the number of AgNOR bearing chromosomes in the karyotype.
b. Increase in transcriptional activity producing more visible argyrophilic cells.
c. Increase in the proliferating rate of cells resulting in large population showing positivity.
d. Tumours with increased AgNOR counts in interphase nuclei are more likely attributable to cellular proliferation than to N ploidy, because diploid cells are tetraploid in G2 phase transiently, resulting in temporary doubling of NOR bearing acrocentric chromosomes as found by Crocker et al (1989). Immediately before and after mitotic division, the NORs disperse and then re-aggregate leading to increase in the number of countable AgNORs in the nuclei.

Ultra structural appearance of Mammalian Nucleoli (Diag – 2)

a. **Granular component :** It is composed of distinct spherical shaped granules each about 15 nm in diameter.
b. **Dense fibrillar component :** It is consisting of tightly packed electron dense 3-5 nm thick fibrils.
c. **Fibrillar centre :** It is consisting of a loose network of fibrils with a little greater average diameter (4-8 nm) than the dense fibrillar component.

Crocker et al (1989), derived procedures of importance followed internationally, for a simple single step colloidal silver staining technique.

The fibrillar centre seen in electron microscopy is the equivalent of the interphase AgNOR seen at the light microscopy level.

History of AgNOR

AgNOR are loops of DNA that encodes for ribosomal RNA production by polymerase I. NOR are argyrophilic, hence detected by silver impregnation technique. Number of AgNORs in a cell nucleus reflects the cell kinetics and proliferative activity.

In rapidly proliferating cells like neoplastic cells, nucleolar disaggregation may take place resulting in dispersion of individual AgNORs.

AgNOR in the field of oncology

Study of AgNORs has started gaining importance in the field of oncology. Dr. J. Crocker and M.J. Egan (1988) in their study of AgNORs in

36 lymphoma cases demonstrated that in non hodgkin's lymphoma, AgNORs diminish in size and increase in number from low grade to high grade malignancy. They may represent elevated transcription leading to more r RNP gene copies with increasing malignancy.

In 1993 Dr. Derenzini D'Trere identified that high number of metaphasic AgNORs in hepatocytes of patients with chronic liver disease is associated with increased risk of hepato cellular carcinoma.

Yamanoto N (1993) studied the NOR proteins in renal cell carcinoma and it's significance as a marker of proliferative activity. The increase in Argyrophilic nucleolar protein of a cell has been shown to reflect the rDNA transcriptional activity and state of cellular activity. The AgNOR values in renal cell carcinomas were higher than those in the normal tubules and the numbers increased with the progress of grades. Moreover, the numbers of NOR, were higher in infiltrating types such as Sarcomatoid type. The patients with renal cell carcinoma having counts of 4.5 or more showed a significantly poor prognosis.

Isaka et al (1993) examined AgNORs in 96 patients with renal cell carcinomas and concluded that the number of AgNORs increased along with upgrading. When AgNORs were compared in various cell types, the number in pleomorphic, spindle cell and Bellini duct carcinoma were more than that in common types.

Shimazui T et al (1995) evaluated the prognostic significance of nucleolar organizing regions in renal cell carcinomas and found that the

NOR indices correlated well with survival curve. The low NOR index group had a survival rate of 100% while in those patients with higher NOR index the mortality was significantly increased. The patients with a low NOR index have a better prognosis than those with a high NOR index within each tumour grade.

V.V. Radhakrishnan and D.Rout (1994) selected 10 cases of Medulloblastoma in which there are difficulties in making histopathological diagnosis as cells of internal granular layer of cerebellum resembled cells of medulloblastoma. By AgNOR there was an average of AgNOR count 0-2/nucleus in cells of internal granular layer and an average of 4.82 AgNORs per nucleus of medulloblastoma cells.

Tannafel A et al (1996) studied the prognostic value of ploidy and proliferation markers in renal cell carcinomas and concluded that cellular proliferation may prove to be another method of predicting biologic aggressiveness. Statistical correlation were seen between tumour grade and AgNOR dots.

Tomobe N et al (1997) stated that AgNOR is a new prognostic indicator for patients with renal cell carcinoma.

Delahuni B et al (1988) assessed the prognostic markers for renal cell carcinoma and evidenced that only nuclear grade was associated with the outcome out of all other prognostic parameters tested in various studies. Only assessment of tumour proliferation markers (Silver staining nucleolar

organiser regions, PCNA and Ki-67antigen) had shown consistent association with survival.

Smith R, Crocker J, in 1988, studied the significance of NOR in breast malignancy, and proved its statistical significance.

In 1989, Giri, DD Nottingham JF, Lowry J et al, correlated AgNOR, in benign and malignant breast lesions.

In 1988, J. Crocker and Paramjithnar from Department of Histopathology, East Birmingham Hospital studied AgNORs in 90 paraffin sections of non hodgkin's lymphomas. A significant difference was found between the number of AgNORs in the nuclei of low grade lymphomas (from the mean of 1 to 1.5 / nucleus) and those of high grade lymphomas (a mean of 4.4-6.8 per nucleus).

AgNOR in Gynaecological Oncology

John Crocker, David, A.R. Boldy and Mark et al in 1988 enumerated standardized approach in counting AgNOR.

Firstly all Silver stained structures could be counted but when lying in groups, each cluster (almost aggregated or partly disaggregated nucleoli) treated as one structure.

Secondly where AgNOR could be counted within a nucleolus each AgNOR could be counted as 1 unit together with the smaller AgNOR seen outside the nucleus. Overall he suggested that total AgNOR dots both intra and extra nucleolar be enumerated.

In 1995, D. Prathiba, Sarah Kuruvilla et al of Sri Ramachandra Medical College and Research Centre, Chennai studied the value of AgNORs in premalignant and malignant lesions of the cervix. The mean AgNOR count was found to increase progressively from normal to CIN I, II, III and invasive carcinoma.

In 1989, Newbotd M, Rollason P, Ward K studied the NOR and proliferative index in glandular and squamous carcinomas of the cervix and found that they have pattern with complete loss of polarity with Significant Prognostic Value – J. Clin. Patho 1989; 42 : 441-2

In 1993, Lakshmi S, Nair SA, Jayashree R et al studied the AgNOR in inflammatory, premalignant and malignant lesions of the cervix and showed gradual increase in NOR count with increasing severity of dysplasia. - Cancer Lett 1993; 71 : 197-201.

In 1997, Agarwal J, Gupta JK studied the difference in NOR's in neoplastic and non neoplastic epithelium of the cervix. - Ind J. patho; 1997;40;125-7.

In 1997, March Calore EE, Made My. Cavaliere MJ of Brasil conducted a study of AgNOR technique in cervical intra epithelial neoplasms and found that AgNOR counting can be useful in the identification and classification of individual cases of intra epithelial neoplasia and their differentiation from difficult cases of cervicitis. - Minerva Gynecol 1997, March 493, 59 to 62.

In 1997, Jyotima agarwal, J.K. Gupta et al of Department of pathology, Kamala Nehru Memorial Hospital, Allahabad found that Mean AgNOR count in CIN and Malignant metaplasia and chronic cervicitis and also adeno carcinomas have higher AgNOR when compared to squamous and adeno squamous carcinoma. - Indian J Pathol and Micro 40 (2) 125-127 (1997).

In 1998 May, Terlikowski S, Lehezewski A, Sulkowski S, Sulkowski M, compared AgNOR counts of CIN II and SIL and suggested that AgNOR counts could be of significance in the evaluation of cervical lesions and could elaborate histopathological diagnosis.

In 2001, J.S. Misra Vinita Das and Madhulika Singh et al of department of O & G King George's Medical University Lucknow, India performed an assessment of AgNOR count as tumour marker in cervical carcinogenesis. It showed importance of AgNOR in discriminating cervical lesions, high AgNOR in follow up study with progression to dysplastic lesion.

In 1999, Kurian, Al Nafussi et al studied the relation between cervical glandular intra epithelial neoplasia to micro invasive and invasive adeno carcinoma of cervix. - J. Clin. Pathol. 1999, 112, 117.

In 2001, Sakai Y, Sakai AT, Isotani S, Caveliere MJ, De Almeid et al of Adoifo latry institute Pathology division Saopauls had done morphometric evaluation of NOR of intra epithelial neoplasia. They found that number of cells with one dot decreased with increasing grade of CIN. Number of cells

with more dots increased with increasing grades of CIN. Total number of dots per 100 cells increased with increasing CIN and concluded that counting the cells with 4 or more dots is the parameter for distinguishing the grade of CIN. - Pathol. Respract 2001, 1997 (3) 189-192.

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In 2003, Rajini Kawshik, Vijaysharma, Archana Gulate RR Sharma et al of Department of Pathology ICMC Shimla studied AgNOR counts in cervical lesions. They found that AgNOR as a reliable indicator of cell proliferation and malignant potential of the lesion and can be used as an adjunct in routine histopathology in the cervical lesion. - Indian J. Pathol & Micro 2003, Vol. 46, No (2) 2001-2003.

In 1998, Rowlands DC studied AgNOR in cervical intra epithelial neoplasia and proved the increase in count as the dysplasia progresses. -J.Clin. pathol. 1998 41; 1200-2.

In 1989, Cullimore JE, Rollason TP, Marshall T studied AgNOR in adenocarcinoma insitu of endocervix and proved the prognostic value. - J. Pathol. 1989; 42: 1276-80.

Egan M, Freeth M, Crocker J studied the relationship between intra epithelial neoplasia of cervix and the size and number of AgNOR dots, size and number increased in advanced grades of intra epithelial neoplasia. - Gyn. Onc. 1990, 36; 30-33.

Kafil Akhtar, Ghazala Mehdi, Veena Maheswari, Shahid Ali Siddiqui : Rajyashri Sharma from Department of Pathology, Radio therapy and

gynaecology, J.N. Medical College, Aligarh Muslim University, Aligarh. conducted a study on diagnostic and prognostic significance of AgNOR counts in radiotherapy treated squamous cell carcinoma of the cervix and concluded that AgNOR is an effective tool, reflecting the proliferation rate of tumour and has a significant diagnostic and prognostic value in tumour pathology. - J. Obstet Gynaecol India W155, No. 2, Pg 163-166. March – April 2005.

The major advantages of AgNOR staining technique

1. Cost and time effectiveness.
2. It can be used on archival as well as fresh tumour material.
3. It can also be observed on smear squash cytologies and all cultures.
4. It does not require special fixatives or complex apparatus.

The major disadvantages are

1. The counting procedures adopted are usually manual and hence long and tedious.
2. Observer error is the major cause of inaccuracy and inconsistency.
3. The dots of AgNOR interphase nuclei need not always correspond actively to the number of such types in the karyotype, as found by Underwood and Giri 98. (Loc cit. 1988)
4. Overlap and coalescence may result in misjudged counts as said by Crocker et al. 22 (Loc cit 1989).

CIN

The epidemiological data correlate well with the current understanding of the pathogenesis of cervical neoplasia. The incidence of cervical dysplasia is reported to be 15 : 1000 in women who were cytologically screened. The incidence of severe dysplasia is reported to be 5 : 1000 according to ICMR studies (Shaw).

The period of early squamous metaplasia is the time of greatest risk for cellular transformation and development of cervical neoplasia. In this period young metaplastic cells have phagocytic properties and if some potent mutagen is present in the vagina during this time, the epithelium might undergo the cellular transformation. The early squamous metaplasia occurs almost exclusively in puberty and early adolescence and in the first pregnancy. Therefore women who begin sexual activity at an earlier age when the metaplastic process is more active, have a greater chance of developing cervical carcinoma.

Age adjusted incidence rates for invasive cancer ranges from 19-44/100,000 women in various cancer registries in India. Higher incidence is noted in Chennai 43.5/1,00,000 followed by 30.1/100,000 at Delhi and 19.4/100,000 at Mumbai.

The cumulative risk of CA cervix is 1.58% in Mumbai and 3.6% in Chennai. In other words, 1 in 62 woman in Mumbai and 1 in 28 woman in Chennai may develop cancer cervix in their life (ICMR – 1992).

Natural History of CIN

Original squamous epithelium is derived from the congenital sinus epithelium, starts at the vulvovaginal line, lines the vagina, covers the major portion of the cervix and abuts upon the columnar epithelium to form the original squamocolumnar junction.

Under low vaginal acidity, the reserve cells proliferate, lifting the columnar epithelium. There is centripetal growth of original squamous epithelium growing beneath the columnar epithelial cells. Process of squamous metaplasia begins in the lips of the columnar villi which are first exposed to vaginal acidity.

A new squamocolumnar junction is continuously formed that gradually replaces the native columnar epithelium. The deeper clefts, however, may not be completely replaced by the metaplastic epithelium, trapped under the squamous epithelium resulting in the formation of Nabothian cysts. Gland openings and nabothian cysts mark the original squamocolumnar junction and the outer edge of the original transformation zone. The area between original squamocolumnar junction and the new squamocolumnar junction is referred to as Transformation Zone (TZ).

Physiologic transition occur during 3 phases of life.

1. Foetal
2. Menarche
3. First Pregnancy

Anatomy of Transformation Zone

Proximal border of transformation zone is the upper limit of squamous metaplasia where the immature squamous metaplasia abuts a circumferential ring of unaltered columnar epithelium.

Original Squamocolumnar junction has 4 layers.

1. **Basal layer** : Single row of immature cells, with large nuclei and small cytoplasm.
2. **Parabasal layer** : Two to four rows of cells that have normal mitotic figures.
3. **Intermediate layer** : Includes four to six rows of cells with large amount of cytoplasm in a polyhedral shape separated by intercellular space.
4. **Superficial layer** : Includes five to eight rows of cells with small uniform pyknotic nuclei and acidophilic cytoplasm filled with glycogen. These cells exfoliate and form the basis for pap smear.

Columnar Epithelium

Single layer of columnar cells with mucus at the top and a round nucleus at the base.

Metaplastic epithelium

Transformation from columnar epithelium to squamous epithelium is known as metaplasia to the histologists and as TZ to the colposcopists. This is the area of development of neoplasia and the area of interest to the colposcopists. It's caudal limit is the original squamocolumnar junction and cephalic limit is the new squamocolumnar junction.

Pathogenesis of CIN

In most cases, CIN is believed to originate as a single focus in the transformation zone at the advancing SCJ. The anterior lip of cervix is twice as likely to develop CIN as the posterior lip and CIN rarely originates in the lateral wall.

Once CIN occurs, it can progress horizontally to involve the entire TZ, but usually does not replace the original squamous epithelium. Proximally CIN involves the cervical clefts and the area tends to have more severe lesions.

CIN is most likely to begin either during menarche or after first pregnancy, when metaplasia is more active. Conversely, a women who has reached menopause without developing CIN has little metaplasia and is at a lower risk.

Progression and Regression of CIN

The spontaneous regression rate of biopsy proven CIN I is 60-85% in prospective studies. This regression typically occurs within a 2 year follow up with cytology and colposcopy..

This information has led to the recommendation that patients who have biopsy diagnosis of CIN I with satisfactory colposcopy and who agree to the evaluation every 6 months can be treated by observation. If the lesion progresses in follow up or persistent after 2 years, ablation is recommended. (Novak's gynaecology)

Etiology and Risk factors

1. Marriage and sexual behaviour

Increased risks are associated with coitus at an early age, coitus with multiple sex partners, and coitus with high risk men (Miller et al 1976, Canadian Task Force – 1982).

2. Local hygiene

Cancer cervix is found to be positively associated with lack of daily genital washing and negatively with the use of clean sanitary napkins during menstruation (Zhang et al, 1989).

3. Contraception

Barrier methods may be protective while OCP increases the risk of CIN and invasive cancer which is attributed to the hormonal changes produced in the cervical epithelium (Beral et al, 1988).

4. Dietary Factors

Deficiency of Vit A, C, E and folic acid are related to cancer cervix and dietary supplementation of these vitamins may prevent its occurrence (Verreault , 1989).

5. Smoking and Douching

Smoking and douching with tar substance may have a role in CIN (Winkelstein et al, 1991). Smoking more than 10 cigarettes per day had increased the relative risk to get CIN and invasive cancer. (Nunez et al, 2002).

6. STD

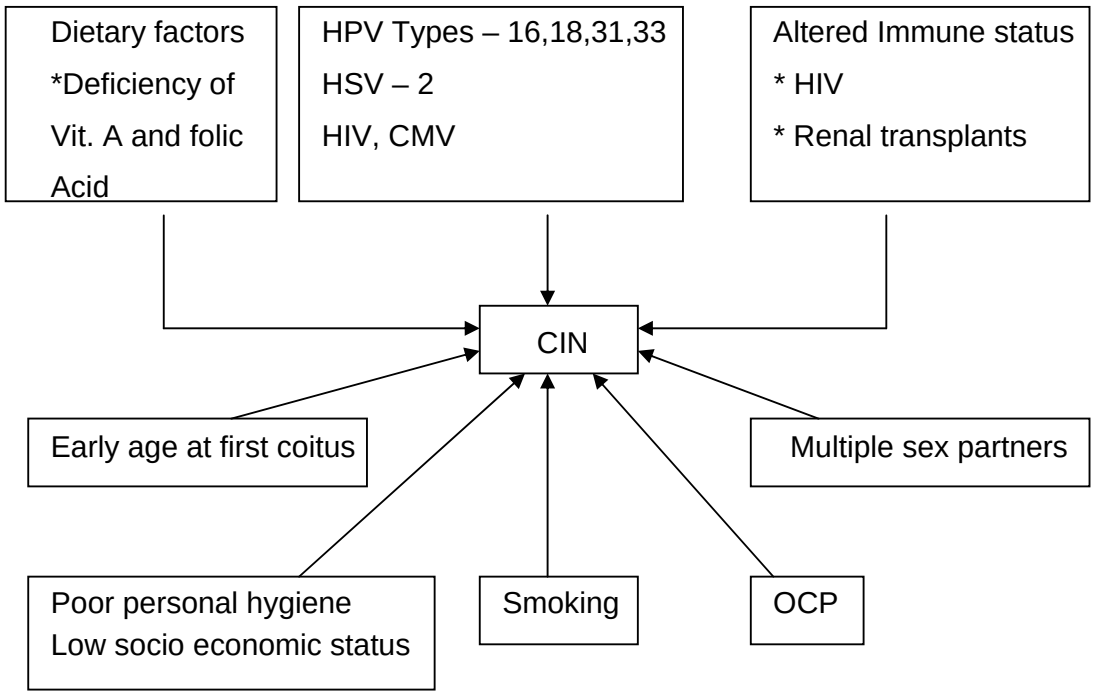
There is a definitive association of Human Papilloma virus (HPV) – 16, 18, 31, 33 with CIN. HPV – 16 is associated with CIN. HPV – 18 is associated with high grade squamous cell carcinoma and HPV – 18 is associated with adenocarcinoma , HPV – 31,33,35 are associated with intermediate oncogenicity (Crum et al, 1985, Levine et al, 1984). Increased risk of cervical cancer has been reported with other STD infections like HSV – 2, HIV and CMV. This risk is found to increase significantly with increase in the number of apparent organisms. (Schmauz et al, 1989)

7. Immunosuppression

Both preinvasive and invasive lesions are more prevalent in chronically immuno compromised women, eg. In HIV (Maimen et al – 1998) and in renal transplants (Alhoub et al – 1988 and Mayernon et al – 1991).

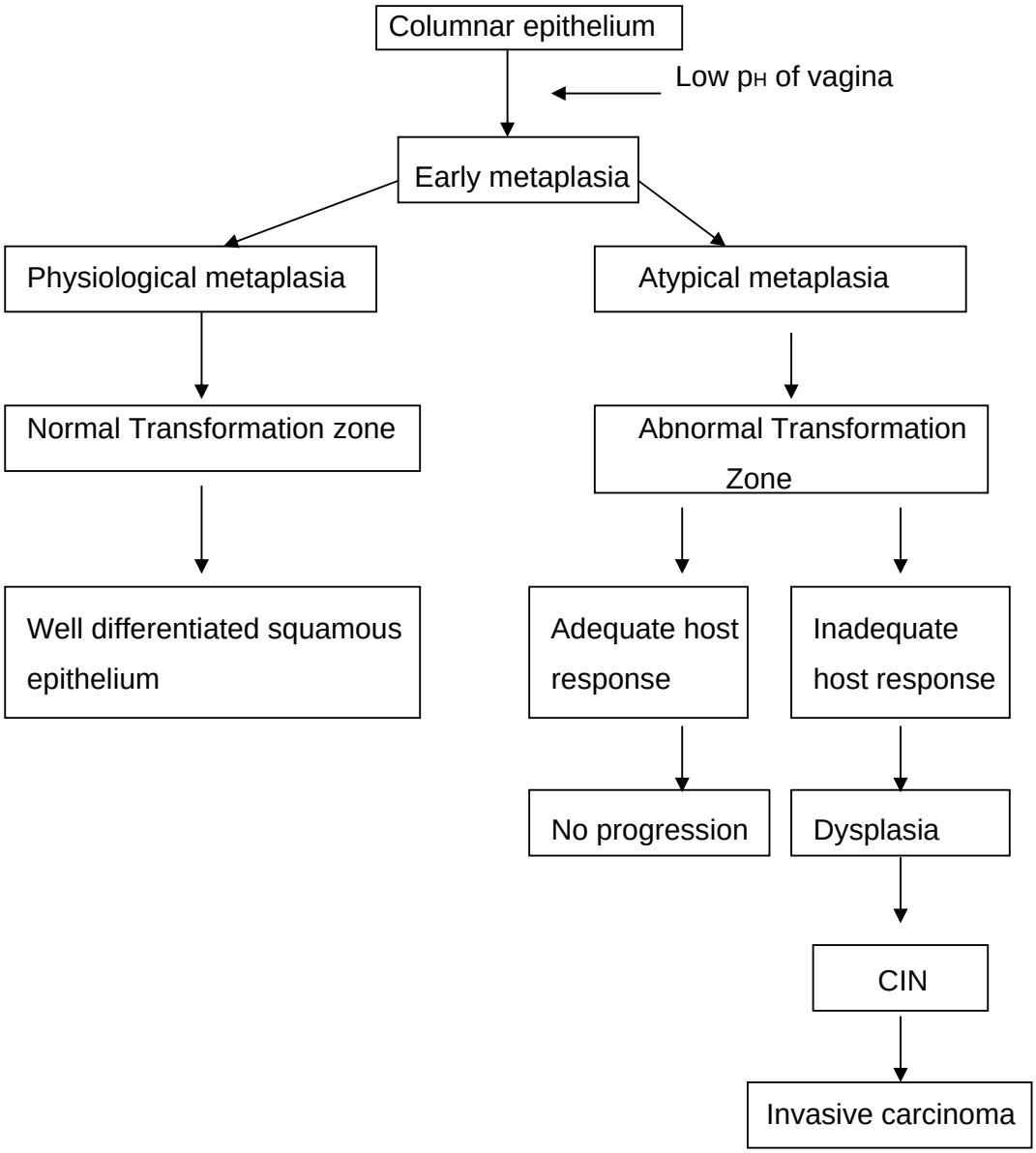
8. Parity

Although multiparity is associated with increased incidence of carcinoma cervix, there is no significant evidence to prove it. (Cramer et al, 1986)



The transformation to premalignant changes is a rapid process that lasts for days or weeks at the most, but the promotion of these changes is a long term process. Some lesions can stay on the cervix indefinitely without change and some can progress quickly to CIN or invasive carcinoma depending on host response.

Scheme of pathogenesis of cervical neoplasia from STAFLA, MATTINGLY RF. AMJ. O.G. 1977



CIN I and CIN II require treatment. This is based on metaanalysis showing that CIN II progresses to CIS in 20% of cases and to invasion in 5%.

CIN	Regress.	Persist	Progress to CIN III	Progress to invasive CA
CIN I	57%	32%	11%	1%
CIN II	43%	35%	22%	5%
CIN III	32%	56%		>12%

(From Ostor AG-Inter. J. Gynaecol. Pathology, 1993; 12)

Precursors of invasive squamous cell carcinoma

The term dysplasia was first introduced by Reagan in 1953. Dysplasia represents a change causing an alteration and disorderly arrangement of the differentiated basal cells of stratified squamous epithelium.

The three degrees of dysplasias mild, moderate and severe according to Novak and Woodruff (1979) are characterised by,

i) Mild

The dysplastic cells extend from one quarter to one third of the way from the basal layer.

ii) Moderate

Cellular aberrations extend through one half to two third of the thickness of epithelial layer.

iii) Severe

The dysplastic cells penetrate through 75-90% of the epithelium.

Histopathology appearance of severe dysplasia resembles carcinoma in situ with the exception that a few cell layers near the surface are still capable of maturation.

Carcinoma in situ is characterized by malignant change involving the whole thickness of the squamous epithelium from the basement membrane to the surface, disclosing an immature disorganized pattern with complete loss of polarity.

Richart (1966) first introduced the term cervical intra epithelial neoplasia (CIN) to denote the ranging degrees of intra epithelial abnormalities. The varying degrees of CIN ranging from mild dysplasia to Carcinoma in situ represents a continuum in the neoplastic process.

Richart (1966) graded the various degrees of CIN into

- ★ CIN I – Mild dysplasia
- ★ CIN II – Moderate dysplasia
- ★ CIN III – Severe dysplasia and Carcinoma in situ

Carcinoma in situ has been grouped under CIN, although histologically there is a slight difference between severe dysplasia and carcinoma in situ.

Therepeutically and prognostically there is hardly any difference between the two.

Feature variations with increasing severity of dysplasia

Decrease	Increase	Varies
Cellular cohesion	Mitosis	Nuclear hypertrophy
Amount of cytoplasm	Nuclear cytoplasmic ratio	Anisokaryosis
Multinucleation	Anisochromatism	Hyperchromatism
Degree of maturation	Nuclear membrane irregularities.	Nucleoli
Normal flora		

Screening Procedures for CIN

1. Pap Smear
2. Colposcopy
3. Cervicography
4. Speculoscopy (VIA, VILI)
5. HPV DNA detection and typing
6. Polarprobe (Truscan)
7. Spectroscopy

Pap Smear

The pap smear initially described by papanicolaou and Traut is used to detect exfoliative cells from the cervix that may be precancerous or cancerous.

Conventional Pap Smear

According to American Cancer Society, ideal time to take pap smear is 5 days after menstruation. The patient should be instructed not to use a vaginal douche or any type of lubricant or spermicide for 24 hours prior to having a cytologic specimen obtained. The ectocervix and the area of vagina adjacent to the cervix must be fully visible when smear is obtained. The patient should not bleed and should not have marked vaginal infection. The infection must be treated accordingly and patient must be rescheduled for pap smear during next cycle.

The smear is taken with Ayer's spatula or with moistened brush around the external os by making 360^0 rotation with minimal pressure and

smeared and fixed in a glass slide with 95% alcohol. Staining done with papanicolaou staining procedure which employ,

- Haemotoxylin as a nuclear stain and
- Orange G – 6 and Eosin alcohol (Eo-36) as cytoplasmic counterstains. The slides are finally mounted in Canada balsam and examined and interpreted.

Various forms of Interpretations

Papanicolaou (1943)	**Richart**	**WHO (1975)**	**Bethesda (1998)**
Gr I – Normal cells	Normal	Negative for cancer	Normal
Gr II – Slightly abnormal, suggestive of inflammatory changes. Repeat smear after treating infection	Negative	Atypical squamous cells	Inflammatory - HPV - ASCUS
Gr III – More, Serious type usually indicative of biopsy	CIN I	Mild dysplasia	Low SIL
Gr IV – Distinctly abnormal and definitely require biopsy	CIN II CIN III	Moderate and severe dysplasia	High SIL
Gr V – SCC	SCC	SCC	SCC

Cervical biopsy

Gold standard confirmatory test. Different types of biopsy methods are done which include punch biopsy, Loop excision biopsy and conisation biopsy. Punch biopsy is the most commonly practiced method but it tends to crush and may not include stroma. A low voltage diathermy loop biopsy

requires more sophisticated equipment but can control haemorrhage and produces samples of greater size. Prendiville et al have shown that the artefactual damage is minimal and a larger biopsy can be taken for the diagnosis of microinvasion or invasion.

Screening Intervals

ACS – American cancer society 2002 guidelines

1. **Age to initiate screening** : Three years after the onset of sexual activity, not later than the age 21.
2. **Screening frequency** : Annually with conventional cytology or every 2 years with liquid based cytology. After the age of 30, women with 3 consequetive normal tests may be screened every 2-3 years.
3. **Screening after hysterectomy** : No cytologic testing after total hysterectomy for benign conditions.
4. **Discontinuation** : After the age 70
5. **Routine screening for HPV infection** : Not yet FDA approved. If approved, conventional or liquid based cytology combined with test for DNA from high risk HPV types should be performed not more often than every 3 years.

Management of CIN

1. Management of Low grade CIN (CIN I)

a) **Expectant Management** :- As 60% of CIN I lesions regress spontaneously, observation and prospective follow up of the patient who is willing to come for regular follow up is adequate. The follow up is done with any of following methods :-

   a. Cytology
   b. Colposcopy
   c. Cervicography
   d. HPV DNA detection and typing

These patients should have repeat assessments at 4 to 6 monthly intervals. The patient can exit the surveillance program once three negative cytological smears are obtained.

b) **Active management** :- It is indicated if

   a. The patient will not adhere to close follow up.
   b. Lesion persists for one year.
   c. Association with high risk HPV is documented.
   d. HIV infected patients.
   e. The patient requests treatment

Ablative techniques for treating CIN

Prerequisites

i) The entire lesion is visualised within the TZ.

ii) There is no suspicion of microinvasive or invasive cancer.

iii) There is no suspicion of endocervical glandular disease.

iv) The cytology and histology should correspond.

v) A high degree of clinical expertise

vi) The patient should be compliant with follow up for atleast three years.

Timing – performed in the postmenstrual period under colposcopic guidance.

Types

1. **Cryotherapy** – Rapid freezing causes crystallization of cell water leading to cell dehydration that causes increase in the concentration of intracellular salts and release of lysosomal enzymes which cause disruption of cell membranes and organelles. Freeze – thaw – freeze technique is used. CO_2 at -60^0c and N_2O at -89^0c are the gases used. Depth of tissue destruction is around 4-5 mm. No anaesthesia is required. Complications like uterine cramping, profuse watery discharge, slight spotting may last for 2-3 weeks. Cure rate is 99%.

2. **Electrocoagulation diathermy (Electrodiathermy)** – This destroys tissue by a combination of fulguration and coagulation. Done under general anaesthesia. Temperatures > 70^0c are produced making this procedure painful. Discharge and bleeding may occur postoperatively. Depth of tissue destruction is atleast 7 mm and cure rate of 88-97% are quoted.

3. **Electro coagulation** – Here, the tissue is destroyed by the application of a thermasound heated to 120^0c to the cervical surface.

Treatments of approximately 20 seconds are given to five overlapping areas. Local anaesthesia is required and cure rate of 94% may be achieved.

4. **Laser vaporization** – Laser energy boils intracellular water, producing steam and exploding the cell. CO_2 laser is most commonly used. Energy density of 20-25 W, power density of 500-1200 w/cm^2 and a beam spot diameter of 1.5 to 2 mm used. Depth of destruction achieved is 6-7 mm and procedure is completed in 15-20 minutes. Advantages are ; depth and extent of tissue destruction can be accurately controlled, rapid healing of margins, minimal bleeding and discharge and TZ will be visualised during follow up.

Management of high grade CIN (CIN 2 and CIN 3)

Exicisonal techniques are preferred. Types of exicisional procedures are:-

1. **Conisation** – Cold knife conisation
2. **LLETZ** – Large loop electro surgical exicision of transformation zone

Indications :-

i) Unsatisfactory colposcopy

ii) Suspected invasion on cytology, colposcopy or biopsy

iii) Endocervical curettage is positive

iv) Lack of correlation between cytology, colposcopy and biopsy

v) Suspected adenocarcinoma

Types of cones – shallow cones where lesion is visible and columnar epithelium is also visible in the cervical canal.

Deep cones when the apex of lesion is in the cervical canal and part of lesion is out of sight. Not performed in woman, desirous of future childbearing.

		Cold knife conisation	**Laser conisation**
1.	Admission and anaesthesia	Needed and GA	Office procedure LA
2.	Colposcope	Not used	Used
3.	Healing process	Slow	Rapid
4.	Scarring and Stenosis	Present (26-36%)	Rare (7%)
5.	Visibility of new SCJ	No	Yes

Depth of tissue destruction in laser conisation is 7-8 mm. Efficacy is 96%.

LLETZ

Indications

i) High grade CIN

ii) CIN I persisting for > 12 months

iii) Glandular intraepithelial neoplasia

Contraindications

i) Extremely large lesions

ii) Vaginal extension

iii) Obvious clinical carcinoma

It is done under local anaesthesia under colposcopic guidance. The loop is advanced into the cervix just lateral to the TZ until the required depth has been achieved. It is then slowly taken across the cervix enveloping the TZ. Fine wire loops 2.5 x 2.5 cm with electrosurgical units with power output of 30 and 60 W units are used. Cure rates for small loops are 80% and for large loops are 90%. The specimen is marked at the endocervical canal margin at 12 o'clock position with a needle.

The main advantages are; Short time, less morbidity and a tissue specimen for pathological examination is available.

Hysterectomy – can be considered for the treatment of CIN in;

1. Poor compliance for follow up.
2. Microinvasion.
3. Positive cone margins.
4. Associated gynaecological problems like fibroid, prolapse.

MATERIALS AND METHODS

MATERIALS AND METHODS

This study was carried out in Department of Obstetrics and Gynaecology, Govt. Rajaji Hospital in collaboration with Department of Pathology, Madurai Medical College in the period of May 2005 to May 2006.

50 patients from gynaec outpatient department and gynaec ward were randomly selected for this study after obtaining informed written consent. Criteria for selecting women for study was the presence of one or more of the factors given below.

Inclusion criteria

1. Age : 20-60 years.
2. Patients with abnormal symptoms like profuse white discharge, post coital bleeding, intermenstrual bleeding or post menopausal bleeding.
3. Patients with clinically unhealthy cervix diagnosed by speculum examination like cervical erosion, Hypertrophy, growth etc.
4. Patients with pap smears showing dysplasia.

Exclusion criteria

1. Women with age more than 60 years and less than 20 years.
2. Pregnant women
3. Women who have not had coitus.
4. Women who underwent cauterization, cryotherapy or radiotherapy.
5. Women who underwent total hysterectomy.

50 patients from outpatient gynaec department and gynaec ward were randomly selected. A detailed history regarding the age, occupation, educational status, socioeconomic status, history of STD, OCP Usage, No. of partners, age at first intercourse, presenting complaints, menstrual history, marital history and obstetric history were recorded. It was followed by speculum examination and bimanual pelvic examination. Cervical biopsy taken from all the patients.

Technique of punch biopsy of cervix

1. Patient was placed in lithotomy position.
2. The labia were separated and a sim's speculam was introduced into the vagina.
3. The cervix was visualized.
4. Punch biopsy was taken using biopsy forceps.

Tray of cervical punch biopsy contains,

1. Sim's speculum
2. Cervical biopsy forceps
3. 10% formalin

Materials for AgNOR staining

1. 5μ thin histopathological sections.
2. 20% aquous silver nitrate solution.
3. 2% gelatin.
4. 1% formic acid.

The specimens were fixed in 10% formalin. After paraffin embedding, 5μ thin sections were made and stained. Apart from the routine hematoxylin and Eosin stain, special stain for AgNOR staining was used.

Each of the samples then subjected to an argyrophilic staining for the nucleolar organizer region according to the modified colloidal silver technique of Crocker et al (Loc cit 1989).

AgNOR Stain

Method of preparing colloidal silver staining solution

It was prepared by mixing 2 volumes of 20% aquous silver nitrate solution (20g/dl) with one volume of 2% gelatin (2 g/dl) in 1% formic acid (1 g/dl).

Staining Procedure

1. 5μ thin sections were cut from the routinely processed, formalin fixed tissue and were de-paraffinised and brought through graded alcohols.
2. The slides were then immersed in distilled water for 10 minutes.
3. The sections were then layered with silver nitrate gelatin formic acid solution.
4. The slides were then incubated in a dark room for 40 minutes at room temperature.
5. The slides were then washed in distilled water dehydrated, cleared and mounted in D.P.X. mountant.

AgNOR Score

The stained slides were viewed under oil immersion and intra nuclear silver dots are hand counted making use of light microscope. After counting atleast 100 cells, AgNOR score was calculated, i.e. Mean number of AgNOR dots per nucleus. The count was repeated by another person to minimize observer error.

Three main types of AgNOR configuration can be described in normal or neoplastic cells. They are shown in the Diagram 3 to 5.

In diagram 3, the NORs are fully aggregated to form a solitary, rounded argyrophil structure, often called an AgNOR but corresponding to the nucleolus.

Diagram 4 shows a nuclear pattern which is often seen in proliferating cells where NORs can be seen within the nucleolus.

Diagram 5 represents the distribution of small true AgNORs throughout the nucleoplasm as frequently observed in highly malignant cells Crocker et al (loc cit 1988).

There are two approaches to count AgNORs

Firstly all silver stained structures could be counted, but when lying in groups each cluster (almost aggregated or partly disaggregated nucleoli) treated as one structure (Type 1 method).

Secondly, where AgNORs can be separately seen within a nucleolus, each AgNOR could be counted as a unit, together with the smaller AgNORs seen outside the Nucleolus (Type 2 method). Type 2 method has been followed in our study.

It has been observed that Type 2 method of counting have higher AgNOR scores than Type 1 method of counting.

RESULTS AND ANALYSIS

RESULTS AND ANALYSIS

A. CHARACTERISTICS OF THE SELECTED CASES

Table 1 : Age

Age group	Cases	
	No.	%
20-29	5	10
30-39	12	24
40-49	16	32
50 & above	17	34
Total	50	100
Mean	43.9 yrs	
S.D.	11.2	

Among the 50 women studied, 10% were between 20-29 years; 24% were between 30-39 years, 32% were between 40-49 years and 34% were 50 years and above.

Table 2 : Education

Education	Cases	
	No.	%
Illiterates	26	52
Up to 10^{th} Std.	17	34
+1, +2, Degree	7	14

Among the 50 women studied, 52% were illiterates; 34% had primary / high school education and 14% had higher education.

Table 3 : S.E. Status

S.E. Status Income Rs. Per month		Cases	
		No.	%
I	< 1000	38	76
II	1001-1500	7	14
III	1500-2000	5	10
IV	> 2000	-	-

Majority (76%) of women belonged to low income group, (< 1000 Rs. Per month).

Table 4 : Parity

Parity	Cases	
	No.	%
1	3	6
2	18	36
3	19	38
4 & above	10	20
Mean	2.7	
S.D.	0.9	

Among the 50 women studied, 6% were para 1; 36% were para 2; 38% were para 3 and 20% were para 4 and above; Majority were para 2 and above.

Table 5 : Duration of marital life

Duration of marital life	Cases	
	No.	%
< 5 yrs	4	8
5-10 yrs	14	28
11-20 yrs	14	28
> 20 yrs	18	36

Among the 50 women studied, 8% have < 5 years of marital life; 28% have 5-10 years of marital life; another 28% have 11-20 years of marital life and 36% have more than 20 years. Majority of women have sexual life of > 20 years.

Table 6 : Multiple Sex Partners

More than one partner	Cases	
	No.	%
Yes	1	2
No	49	98

Among the study group, 2% had contact with more than one partner.

Table 7 : Contraception

Contraception	Cases	
	No.	**%**
Barrier	2	4
OCP	3	6
IUCD	6	12
Permanent	21	42
Nil	18	36

Among the 50 women studied, 4% practiced barrier method, 6% were taking OCP; 12% of women had IUCD inserted; 42% were permanently sterilized and 36% of women did not practice any form of contraception; In our study group, majority of women were permanently sterilized.

Table 8 : Complaints

Complaints	Cases	
	No.	**%**
Bleeding		
Present	22*	44
i. Post coital bleeding	4	8
ii. Post menopausal bleeding	13	26
iii. Inter menstrual bleeding	5	10
White Discharge		
Present	31*	62
****Both bleeding & white discharge***	3	6

The common complaints, the woman presented with were either white discharge or bleeding pv 62% presented with white discharge. 44% presented with bleeding, out of which 8% postcoital, 26% postmenopausal and 10% inter menstrual bleeding. 3 patients presented with both the complaints.

Table 9

Previous History of STD and Screening

	Cases	
	No.	**%**
H/O STD		
a) Present	-	-
b) Absent	50	100
Previous screening		
a) Present	-	-
b) Absent	50	100

Among the study group, none of them gave proper H/O STD in the past or previous screening.

Table 10

Clinical appearance of cervix by speculum examination

	Cases	
	No.	**%**
I. Erosion		
a) Present	25	50
b) Absent	25	50
II. Growth		
a) Present	19	38
b) Absent	31	62
III. Hypertrophy		
1. Present	4	8
2. Absent	46	92
IV Ulcer		
1. Present	2	4
2. Absent	48	96

When cervix was visualized using a speculum, the appearance were erosion in 50%, hypertrophy in 8% and growth in 38% and Ulcer in 4%.

Table 11

Histopathological examination

	No.	%
Chronic cervicitis	21	42
Metaplasia	1	2
Mild dysplasia	4	8
Severe dysplasia	4	8
Well differentiated squamous cell carcinoma	4	8
Moderately differentiated squamous cell carcinoma	14	28
Adeno carcinoma	2	4

Among the studied 50 cases with abnormal symptoms, HPE showed chronic cervicitis in 42% of cases, mild dysplasia in 8%, severe dysplasia in 8%, metaplasia in 2% , well differentiated squamous cell carcinoma in 8% of cases, moderately differentiated squamous cell carcinoma in 28% of cases and adeno carcinoma in 4% of cases.

B. CHARACTERISTICS AND HPE FINDINGS

1. Age and HPE Findings

Table 12

Characteristics	HPE Findings													
	Chro. Cervicitis		Metaplasia		Mild dysplasia		Severe dysplasia		SCC. Well differentiated		SCC.mod. differentiated		Adeno carcinoma	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Age														
20 - 29	2	9.5	-	-	1	25	1	25	-	-	1	7.1	-	-
30-39	5	23.8	1	100	3	75	2	50	1	25	-	-	1	50
40-49	10	47.6	-	-	-	-	-	-	1	25	5	35.7	1	50
50 & above	4	19	-	-	-	-	1	25	2	50	8	57.1	-	-
Total	21	100	1	100	4	100	4	100	4	100	14	100	2	100
Mean	40.5		30.0		34.0		39.3		48.0		51.2		40.0	
S.D.	7.7		-		6.9		14.3		14.7		10.5		10	
'p'	0.0001													

Chronic cervicitis was reported in the all age groups from 20 to 50 years and above. 47.6% of patients (10/21) belonged to the age group 40 to 49 years.

Metaplasia was reported in 1 patient in the age group 30 to 39 years.

Mild dysplasia was seen mostly in the age group 30 to 39 years. A few (25%) were in the age group 20 – 29 years.

Severe dysplasia (75%) was mainly reported in the age group 20 – 39 years.

Well differentiated carcinoma was seen in the age group 30 to 50 years and above.

Moderately differentiated carcinoma was seen mainly (92.9%) above 40 years. Only one patient below the age of 30 was reported to have moderately differentiated carcinoma.

Only two patients were reported to have adenocarcinoma and fell in the 30 – 49 years age group.

2. Education and HPE Findings.

	HPE Findings													
	Chro. Cervicitis		Metaplasia		Mild dysplasia		Severe dysplasia		SCC. Well differentiated		SCC.mod. differentiated		Adeno carcinoma	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Education														
Illiterates	10	47.6	-	-	1	25	2	50	2	50	10	71.5	1	50
Upto 10th Std	8	38.1	-	-	2	50	1	25	2	50	3	21.4	1	50
+1, +2, Degree	3	14.3	1	100	1	25	1	25	-	-	1	7.1	-	-

There was not much significant difference between illiteracy and literacy in the distribution of benign and malignant lesions in our study.

3. Socioeconomic status and HPE Findings

S.E. Group	HPE Findings													
	Chro. Cervicitis		Metaplasia		Mild dysplasia		Severe dysplasia		SCC. Well differentiated		SCC.mod. differentiated		Adeno carcinoma	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I	12	57.1	1	100	3	75	3	75	4	100	14	100	1	50
II	5	23.8	-	-	1	25	1	25	-	-	-	-	-	-
III	4	19	-	-	-	-	-	-	-	-	-	-	1	50

Chronic cervicitis, dysplasia and malignancy were more prevalent in the lower income group.

4. Parity and HPE Findings

	HPE Findings													
	Chro. Cervicitis		Metaplasia		Mild dysplasia		Severe dysplasia		SCC. Well differentiated		SCC.mod. differentiated		Adeno carcinoma	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Parity														
1	1	4.8	-	-	-	-	-	-	-	-	1	7.1	1	50
2	9	42.9	-	-	1	25	1	25	2	50	4	28.6	1	50
3	7	33.3	1	100	3	75	2	50	1	25	5	35.7	-	-
$\geq$ 4	4	19	-	-	-	-	1	25	1	25	4	28.6	-	-

Chronic cervicitis was more prevalent in multipara (42.9 + 33.3 + 19 = 95.2%) than in primipara (4.8%). Similarly dysplasia were also more prevalent in multipara (25 + 50 = 75%)

5. Duration of Marital Life and HPE findings

	HPE Findings													
	Chro. Cervicitis		Metaplasia		Mild dysplasia		Severe dysplasia		SCC. Well differentiated		SCC.mod. differentiated		Adeno carcinoma	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Duration of marital life														
< 5 yrs	2	9.5	-	-	-	-	1	25	-	-	1	7.1	-	-
5-10 yrs	7	33.3	1	100	3	75	1	25	-	-	2	14.2	-	-
11-20 yrs	6	28.6	-	-	-	-	1	25	2	50	5	35.7	-	-
> 20 yrs	6	28.6	-	-	1	25	1	25	2	50	6	42.7	2	100

Sexual activity is associated with the changes in the cervix – inflammatory changes ie chronic cervicitis was seen in women who exposed to sexual activity for more than 5 years duration.

Similarly in those with dysplasia and invasive carcinoma, majority (75 – 93%) had a sexual life of more than 5 years duration.

6. Multiple sex partners and HPE findings

	HPE Findings													
	Chro. Cervicitis		Metaplasia		Mild dysplasia		Severe dysplasia		SCC. Well differentiated		SCC.mod. differentiated		Adeno carcinoma	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Yes	1	4.8	-	-	-	-	-	-	-	-	-	-	-	-
No	20	95.2	1	100	4	100	4	100	4	100	14	100	2	100

Only 4.8% of patients with chronic cervicitis came out with the history of having multiple sex partners. Contradictorily such a history was not present in those patients with malignancy or dysplasia.

7. Contraception and HPE findings

	HPE Findings													
	Chro. Cervicitis		Metaplasia		Mild dysplasia		Severe dysplasia		SCC. Well differentiated		SCC.mod. differentiated		Adeno carcinoma	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Barrier	1	4.8	1	100	-	-	-	-	-	-	-	-	-	-
OCP	2	9.5	-	-	-	-	-	-	-	-	1	7.1	-	-
IUCD	4	19	-	-	1	25	-	-	1	25	-	-	-	-
Permanent	8	38.1	-	-	2	50	2	50	2	50	6	42.9	1	50
Nil	6	28.6	-	-	1	25	2	50	1	25	7	50	1	50

OCP use was not associated with dysplasia or Adenocarcinoma. Only 9.5% of patients (2/21) with chronic cervicitis and 7.1% of patients (1/14) with moderately differentiated carcinoma gave the history of OCP usage.

8. Complaints and HPE findings

	HPE Findings													
	Chro. Cervicitis		Metaplasia		Mild dysplasia		Severe dysplasia		SCC. Well differentiated		SCC.mod. differentiated		Adeno carcinoma	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Complaints														
Bleeding only Present	-	-	-	-	-	-	1	25	4	100	12	85.7	2	100
White discharge only Present	21	100	1	100-	4	100	2	50	-	-	-	-	-	-
Both bleeding and white discharge	-	-	-	-	-	-	1	25	-	-	2	14.2	-	-

In all the patients with chronic cervicitis and mild dysplasia the only complaint was white discharge pv. In all the patients with invasive carcinoma the main complaint was bleeding pv.

In severe dysplasia 50% gave complaint of white discharge 25% gave the history of bleeding and 25% gave the history of both.

9. Clinical appearance of cervix and HPE findings

	HPE Findings													
	Chro. Cervicitis		Metaplasia		Mild dysplasia		Severe dysplasia		SCC. Well differentiated		SCC.mod. differentiated		Adeno carcinoma	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Erosion														
Present	17	81	1	100	3	75	4	100	-	-	-	-	-	-
Absent	4	19	-	-	1	25	-	-	4	100	14	100	2	100
Growth														
Yes	-	-	-	-	1	25	-	-	4	100	12	85.7	2	100
No	21	100	1	100	3	75	4	100	-	-	2	14.3	-	-
Hypertrophy														
Yes	4	19	-	-	-	-	-	-	-	-	-	-	-	-
No	17	81	1	100	4	100	4	100	4	100	14	100	2	100
Ulcer														
Yes	-	-	-	-	-	-	-	-	-	-	2	14.3	-	-
No	21	100	1	100	4	100	4	100	4	100	12	85.7	2	100

In 81% of patients with chronic cervicitis (17/21), 75% of patients with mild dysplasia (3/4) and 100% of patients with metaplasia and severe dysplasia – speculum examination showed erosion cervix. In rest of the chronic cervicitis patients, the cervix appeared normal.

85.7% of invasive cancers (12/14) presented with a friable growth. 14.3% of patients with invasive carcinoma (2/14) showed an ulcerative growth which bled on touch.

C. RELATIONSHIP BETWEEN CHARACTERISTICS OF CASES AND AGNOR SCORES

1. Age and AgNOR Score

Age group	AgNOR Score	
	Mean	**S.D.**
20-29	3.6	2.6
30-39	3.08	1.62
40-49	4.06	1.91
50 & above	5.41	2.06
Total	4.24	2.1
'p'	0.0233 (Significant)	

Among the various age groups, there exist statistically significant difference in AgNOR scores. As the age increases, the proliferative activity of cells also increases; it is expressed as increase in AgNOR counts.

Table 2 : Education and AgNOR Score

Education	AgNOR Score	
	Mean	**S.D.**
Illiterates	4.77	2.16
Up to 10^{th} Std.	3.82	1.91
+1, +2, Degree	3.29	1.98
'p'	0.16315 (Not Significant)	

Educational status of the patients and the AgNOR score do not have any significant relationship.

Table 3 : S.E. Status and AgNOR Score

S.E. Status	AgNOR Score	
	Mean	**S.D.**
I < 1000 Rs. / month	4.58	2.16
II 1001-1500	3.29	1.38
III 1500-2000	3.0	1.73
IV > 2000	-	-
'p'	0.1715 (Not Significant)	

S.E. Status does not have a statistically significant relationship with AgNOR score.

Table 4 : Parity and AgNOR Score

Parity	AgNOR Score	
	Mean	**S.D.**
1	4.67	2.52
2	4.17	2.18
3	4.05	2.15
4	4.6	1.72
'p'	0.5371 (Not Significant)	

Parity of the patient and the AgNOR score are not significantly related.

Table 5 : Duration of marital life and AgNOR Score

Duration of marital life	AgNOR Score	
	Mean	**S.D.**
< 5 yrs	3.75	2.22
5-10 yrs	2.93	1.54
11-20 yrs	4.64	2.21
> 20 yrs	5.06	1.98
'p'	0.0194 (Significant)	

Patients with longer duration of married life (> 10 years) have higher AgNOR score than recently married patients and this difference is statistically significant.

Duration of marital life has important role in the development of dysplasia and carcinoma. As the duration increases the chance of developing dysplasia and carcinoma also increases.

Table 6 : Multiple Sex Partners and AgNOR Score

More than one partner	AgNOR Score	
	Mean	**S.D.**
Yes	3.0	-
No	4.27	2.11
'p'	0.6974 (Not Significant)	

Having multiple sex partners does not significantly affect AgNOR score.

Table 7 : Contraception and AgNOR Score

Contraception	AgNOR Score	
	Mean	S.D.
Barrier	3.0	-
OCP	3.5	3.0
IUCD	2.67	1.75
Permanent	4.43	2.06
Nil	4.78	1.93
'p'	0.2197 (Not Significant)	

Contraception status of the patients is not significantly related to AgNOR score.

Table 8 : Complaints and AgNOR Score

Complaints	AgNOR Score for cases			
	Present		Absent	
	Mean	S.D.	Mean	S.D.
Bleeding	6.04	1.22	2.7	1.29
White discharge	3.0	1.59	6.26	0.87

Patients with bleeding have higher AgNOR score than those with white discharge, as the carcinoma presents with bleeding.

Table 9 : Clinical appearance of cervix and AgNOR Score

Clinical appearance of cervix	AgNOR Score for cases			
	Present		Absent	
	Mean	S.D.	Mean	S.D.
Erosion	2.92	1.5	5.56	1.76
Growth	6.05	1.08	3.13	1.76

Patients with Erosion cervix have lower AgNOR than those without Erosion, patients with Growth have higher AgNOR score than those without it. These differences are also statistically significant.

Table 10 : HPE Findings and AgNOR Score

HPE Findings	No. of patients	AgNOR Score	
		Mean	S.D.
Chronic Cervicitis	21	2.48	1.12
Mild dysplasia	4	2.5	0.58
Severe dysplasia	4	5.5	0.58
Metaplasia	1	2.0	-
Sec. Well differentiated	4	6.0	0.82
Sec. Moderately differentiated	14	6.36	0.93
Adeno carcinoma	2	6.5	0.71
Total	50	4.24	2.1
'p'	0.0001 (Significant)		

The various HPE findings exhibit statistically significant difference in AgNOR values. Chronic cervicitis, metaplasia, and mild dysplasia cases have very low scores and the other cases have higher AgNOR scores.

The higher score was present in adeno carcinoma, followed by moderately differentiated and then well differentiated squamous cell carcinoma.

DISCUSSION

DISCUSSION

Cervical cancer is the second most frequent cancer worldwide, in women after breast cancer. However, invasive cancer of cervix is considered to be a preventable condition as it is associated with a preinvasive stage (CIN) making it amenable to screening and treatment.

A study was conducted in the Gynaecology out patient department of GRH between the period May 2005 to May 2006. 50 Women with abnormal symptoms of white discharge and bleeding were screened for the presence of cervical pathology. A detailed history was elicited and a thorough clinical examination was done. Cervical biopsy was taken in all the 50 patients and the sections were stained with conventional H&E stain to study the HPE and with silver stain to study the AgNOR scores. AgNOR score was correlated with HPE report.

Regarding age distribution, high incidence of chronic cervicitis (70%) was seen in the age group 30-49. Shalini et al showed that 32 year is the mean age of patients with benign pathology in her study.

Dysplasia was found to be more common between 20-39 years. Kushtagi and Fernandes in their study showed the prevalence of dysplasia was higher in women over 30 years. Vaidya showed in his study that CIN was more prevalent in the age group of > 35 years.

Squamous cell carcinoma was found to be more common between 40-60 years. Shalini et al showed 25% of well differentiated and 35% of

moderately differentiated carcinomas in the age group 40-49; 50% of well differentiated and 57% of moderately differentiated carcinoma were in age group > 50. The mean age of patient with cancer cervix was 41.

Regarding educational status, abnormal cervical lesions like chronic cervicitis, dysplasia and malignancies were more common among illiterates > 50% of cancers and > 25% of dysplasias were seen in illiterates. This was attributed to lack of awareness of symptoms and failure to seek medical care.

Socio economic status had always been playing an epidemiological role in genesis of dysplasia. In our study, chronic cervicitis, dysplasia and invasive cancers were more common in low income group, 75% of severe dysplasia and 100% invasive cancers were seen in low income group.

Vaidya had showed that low socio economic status had a definite role in the development of dyskaryosis. In his study 80% of CIN I and 50% of CIN II were from low income group. Poor personal hygiene, poor living conditions, unstable marriages and early age at first intercourse are factors associated with both low socio economic conditions and cervical cancer.

Regarding parity, our study showed increased incidence of dysplasia and invasive carcinomas among multiparous women. In patients with severe dysplasia 25% were P2, 50% were P3, 25% were P4 & above. Among invasive cancer patients, 50% were P2, 35% were P3 and 30% were P4 and more.

Similar study by Shalini et al showed the mean parity was 4.2 in patients with invasive cancer. Kushtagi and Fernandez showed the prevalence of CIN was significantly higher in parity of more than 2. Vaidhya also showed more positive cases of CIN with parity more than 4. This might be attributed to hormonal and nutritional changes that occur in pregnancy, immunosuppression during pregnancy and cervical trauma during vaginal delivery (Becker et al and Adadevoh et al).

Duration of marital life that is duration of exposure to sexual intercourse has a distinct role in genesis of cervical dysplasia and carcinoma. In our study, incidence of dysplasia was 25-75% in women with marital life of 5-20 years. Incidence of squamous cell carcinoma was about 50% in patients with marital life more than 20 years. Kushtagi et al had demonstrated the severity of underlying CIN increased with increase in the duration of marital life and hence the increase in the duration of sexual intercourse.

Increasing number of sexual partners had the effect on increasing the risk of developing dysplasia and invasive disease. In our study only 2% of women revealed the history of more than 1 partner, out of which 4.8% had chronic cervicitis.

The relationship between oral contraceptives and development of CIN had been investigated by IARC – International Agency for Research in Cancer and they concluded that the use of OCP increased the risk of CIN upto 4 fold after 5 or more years, among the HPV DNA positive women. In

our study 9.5% of patients with chronic cervicitis used OCP. But non of those with invasive cancer or dysplasia used OCPs

Chronic cervicitis was more common in patients using IUCD and OCP apart from permanent sterilization. Among the chronic cervicitis patients, 28.6% were not using any methods.

25% of patients with mild dysplasia used IUCD 50% of mild and severe dysplasia patients were permanently sterilized; 25% of mild and 50% severe dysplasia patients were not using any methods. 50% of patients with squamous cell carcinoma were permanently sterilized or non users of contraceptive methods.

Prospective studies by Sten et al in los angeles suggested an increased risk of progression of cervical dysplasia among the users of hormonal contraceptives. Vaidya et al in their study showed 40% risk of CIN I in women who had depot provera injection. According to Duggan, OCP produce progression to CIN, by regulating oncogenic sequence of HPV. Since our study sample was small we could not prove it.

Among the complaints, white discharge was present in majority of women with chronic cervicitis, dysplasia and metaplasia. Excessive vaginal discharge playing a role in contributing to the development of CIN was also proved to be a risk factor in the study conducted by Vaidya et al. In their study 24% had vaginal discharge.

Post coital bleeding was found in 50% of patients with severe dysplasia. Shalini R Amita S, in their study, showed the relationship of post coital bleeding and CIN. In their study, among the women who had post coital bleeding, 85.5% had benign findings; 5-6% had HPV and CIN I; 3.6% had CIN II and III and 5.5% had invasive cancer. There was no correlation between the duration of bleeding and pathology.

Regarding the clinical appearance of cervix, the most common finding was erosion in patients with chronic cervicitis (81%) and mild dysplasia 75%. In all patients with severe dysplasia, cervix was badly eroded and bled on touch. Patients with invasive squamous cell and adeno carcinomas presented with a friable growth or ulcer.

The expression AgNOR designates the silver stained NOR proteins. The AgNOR technique is remarkably specific for detection of NORs by virtue of silver binding to a wide array of NOR associated proteins (NOR Aps)

The AgNOR technique stains the NORs as black dots in the nucleus.

The number and size of the NOR dots in malignant cells is significantly different from that in normal or benign cells and reflects the current phase of transcription of cells. Marked cellular atypia is present in repair and regeneration of squamous and columnar epithelia. Cells from epithelial repair have enlarged nuclei which vary in size and shape. AgNOR technique provides an index of cell proliferation. The number, shape and distribution of AgNOR dots counted in the cell gives information not only about the morphology but also about the behaviour of the cell. There is

progressive increase in the mean AgNOR count in cells with squamous metaplasia and in cells undergoing repair upto various grades of CIN demonstrating ongoing proliferation. In the present study it was observed that the mean AgNOR count per cell increases from metaplasia to carcinoma.

In our study there was no significant difference in AgNOR counts between squamous metaplasia and chronic cervicitis. The mean number of AgNORs per nucleus was significantly higher in dysplasia (mild – 2.5$\pm$0.58, severe – 5.5 $\pm$ 0.58 and malignant lesions (squamous cell well diff. – 6$\pm$0.82, mod. Diff. 6.3$\pm$0.93, adeno carcinoma – 6.5$\pm$0.71) as compared to metaplasia – 2 and chronic cervicitis 2.5$\pm$1.15. All carcinomas and severe dysplasia had significantly higher AgNOR counts per nucleus compared to mild dysplasia. NOR counts were significantly higher in adeno carcinoma when compared to squamous cell carcinoma. Statistical analysis revealed significant difference between mean AgNOR counts of chronic cervicitis and dysplasia; mild dysplasia and severe dysplasia, severe dysplasia and invasive carcinoma, squamous cell carcinoma and adeno carcinoma.

Overall the mean AgNOR counts for carcinoma cervix were increased from chronic cervicitis and dysplasia.

The differences in AgNOR count between mild dysplasia and severe dysplasia were statistically significant.

Eagan et al observed that mean AgNOR count increased steadily whereas the mean size of AgNORs decreased from CIN I to CIN II. Cardillo

studied AgNOR counts in cervical smears of squamous metaplasia and cervical intra epithelial neoplasia. The smears previously stained with papanicolaou technique were destained and restained with AgNOR silver. He found statistically significant difference ($p < 0.05$) in AgNOR counts in squamous metaplasia and various grades of CIN.

An Indian study done by Prathiba and Kuruvilla (1995) on the role of AgNOR in diagnosis of premalignant and malignant lesions of the cervix, showed that mean AgNOR count progressively increased from normal to CIN I, CIN II, CIN III and invasive carcinoma. The difference between counts in CIN I and CIN II and invasive carcinoma was statistically significant.

However Rowland in a study on nucleolar organizing regions in cervical epithelial neoplasia, did not find any significant differences in AgNOR count / in squamous epithelium of normal cervix; CIN I and CIN II but there was a small but significant increase in CIN III group; the table shows mean AgNOR counts in various grades in different studies.

Comparative studies

Studies	CC	Metaplasia	CIN I, II (mild Dysplasia)	CIN III (Severe)	Well Scc	Poorly / mod. Diff. Scc	Adeno Ca.
Prathiba Karuvilla, 1995	-	-	1.8	3	4.3	Could not be counted	-
Rowlands 1998	-	-	2.2	2.8	-	-	-
Crocker et al, 1990	-	-	2.9	4.7	-	-	-
Iyomita Agarwal, IK Gupta, 1997	1.56±.42	1.74±.32	3.5±0.04	5.1	5.27±.65	5.37	6.4
Rajnikaushik study, 1999	2.3	-	3.8	5.1	-	6.4	6.7
Present study	2.5±1.15	2	2.5±0.58	5.5±0.58	6±0.82	6.36±0.93	6.5±0.71

Crocker et al in 1990 also showed statistically significant difference in AgNOR counts between CIN I, CIN II and CIN III.

In Jyomita Agarwal, JK Gupta study (1997) AgNORs were counted in biopsies from 202 cases of various lesions and cervix. The mean number of AgNORs per nucleus was significantly higher in CIN (4.05±0.04) and Malignancy (5.50±0.65) as compared to squamous metaplasia (1.74±0.32) and chronic cervicitis (1.54±0.42). Adeno carcinomas had higher AgNOR counts as compared to other carcinomas. It concluded that the estimation of

AgNORs can be helpful in distinguishing benign lesions from CIN and malignancy of the cervix.

In Rajni Kaushik, study, 1995 the mean AgNOR counts in cervical epithelium showed a progressive and statistically significant increase from normal to chronic cervicitis to CIN I, II and III ($p < 0.001$). Scores in carcinoma also exceeded that of CIN ($p < 0.05$). It concluded that this can prove to be a useful adjunct to routine histopathology to evaluate cervical lesions.

Although variations in different studies, the difference is statistically significant in various grades of CIN in all studies except in the study reported by Rowlands.

In our study it was also noted that the size of AgNOR dots decreased with increase in AgNOR count. This is in accordance with the study reported by Eagan who noted an inverse relationship between AgNOR numbers and sizes, and proved that severe dysplasia could be distinguished from mild dysplasia on the basis of AgNOR size.

SUMMARY

SUMMARY

The study was conducted in the Department of obstetrics and Gynaecology between the period May 2005 to May 2006. 50 women with abnormal symptoms were screened for the presence of cervical pathology. Ectocervical biopsy was taken in all the patients. Tissue sections were stained with H&E stain for histopathology and with silver stain for AgNOR counts. AgNOR score was correlated with HPE report.

71.4% (15/21) of chronic cervicitis, 50% (2/4) of well differentiated carcinoma, 35.7% (5/14) of moderately differentiated squamous cell carcinoma and all (100%) adenocarcinoma were reported in the age group 30-49 yrs. All mild dysplasia (100%) and 75% (3/4) of severe dysplasia were found in the age group 20-39 yrs.

Distribution of cervical lesions was not affected by the educational status.

In addition to 80.9% of chronic cervicitis, all dysplasia and Malignancy were seen in low income group (100%).

Chronic cervicitis (95%) and dysplasia (100%) were more commonly seen in Multipara than in Primipara.

90% of chronic cervicitis, 100% of dysplasia and 92.9% invasive carcinoma were seen in patients with more than 5yrs of sexual life.

Sexual promiscuity was present in only 4.8% (1/21) of patients with chronic cervicitis.

Adeno carcinoma and dysplasia were not associated with OCP usage. Only 9.5% (2/21) of patients with chronic cervicitis and 7.1% (1/14) of patients with moderately differentiated squamous cell carcinoma used OCP.

In all the patients with chronic cervicitis and mild dysplasia, the only complaint was white discharge pv. In invasive carcinoma (85-100%) the main complaint was bleeding pv. 50% of severe dysplasia were presented with white discharge and 25% were with bleeding pv. The remaining presented with both.

81% (17/21) of patients with chronic cervicitis, 75% (3/4) of patients with mild dysplasia and 100% of patients with metaplasia and severe dysplasia showed erosion on speculum examination.

85.7% (12/24) of Invasive carcinoma presented with friable growth. Ulcerative growth was seen in 14.3% (2/14) of patients with invasive carcinoma.

As the age increases, AgNOR score also increases.

Educational status, socio economic status and parity of the patients are not significantly related to AgNOR scores.

Patients with long marital life (> 10years) have higher AgNOR score than recently married.

Sexual promiscuity and contraception are not significantly related to AgNOR score.

Patients with bleeding have higher AgNOR score (6.04) than those with white discharge (3).

Patients with growth have higher AgNOR score (6.05) than those with erosion (2.92).

Chronic cervicitis, metaplasia and mild dysplasia cases have low scores.

The higher score was seen in adenocarcinoma (6.5 $\pm$ 0.71) followed by moderately differentiated (6.36 $\pm$ 0.93) and then well differentiated (6 $\pm$ 0.82) squamous cell carcinoma.

CONCLUSION

CONCLUSION

Earlier diagnosis of cervical dysplasia in adult women is a desirable goal. Cervical dysplasia lesions and early invasive cancers should be diagnosed in an earlier stage for instituting appropriate management.

Invasive cancer of cervix is considered to be preventable, since it is associated with a long preinvasive stage (CIN), making it amenable to screening and treatment.

1. In the present study there is a significant difference in AgNOR counts between carcinoma cervix, dysplasia and chronic cervicitis. The mean AgNOR counts in cervical epithelium showed a progressive and statistically significant increase from chronic cervicitis, dysplasia, squamous cell carcinoma and adeno carcinoma. These findings strongly support the view that the proliferative activity of the lesion increases as the malignant potential of a lesion increases. Hence AgNOR scoring can be used as a reliable indicator of cell proliferation, in turn malignant potential of a lesion.

2. AgNOR counts can be useful in differentiating doubtful cases of CIN. It is easy to perform, does not require an elaborate set up and is fairly economical which can be performed routinely in a histopathological laboratory. Another advantage of AgNOR counts lies in retrospective study. The samples can be destained and restained, when unstained slides are unavailable and also in doubtful cases whose corresponding histologic specimens are not available. It also has prognostic significance, it has been

noted that CIN lesions with low AgNOR counts are more likely to regress in comparison to CIN lesions with high AgNOR counts.

3. The findings in this study indicate that AgNOR technique can be used as an adjunct to routine histopathological examination of lesions of the cervix especially in dysplasia. Although more studies are necessary, our preliminary study indicates the potential diagnostic importance of AgNOR counts in cervical cancer and other cervical lesions.

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PROFORMA

PROFORMA

Serial No. :
Name :
IP/ OP No. :
Age :
Income :
Education :

I. Complaints

1. Bleeding PV
   - Post coital bleeding
   - Continuous / Intermittent
   - Intermenstrual
   - Post menopausal
   - Associated pain
2. Vaginal Discharge
   - Duration
   - Quantity
   - Colour
   - Foul smell
   - Pruritis
3. Lower abdominal pain
4. Urinary symptoms
5. Bowel symptoms
6. Loss of weight
7. Loss of appetite

II. Personal History

- ★ Diet
- ★ Hygiene

III. Menstrual History

- ★ Age at menarche
- ★ Cycles
- ★ LMP
- ★ Age at menopause

IV. Married since

V. Sexual promiscuity

- ★ Multiple sexual partners
- ★ History of STD
  - Husband
  - Wife

VI. Obstetrical History

- ★ P L A
- ★ LCB
- ★ Contraception
  - Barrier
  - OCP
  - IUCD
  - Permanent
  - Nil

VII. General Examination

Built	Thyroid
Anaemia	Breast
Pedal edema	BP
Lymphadenopathy	PR
CVS	RS

VIII. Per abdomen

IX. Local examination of Genitalia

- ★ Normal
- ★ Abnormal

X. Speculum Examination

- ★ Cervix
- ★ Vagina

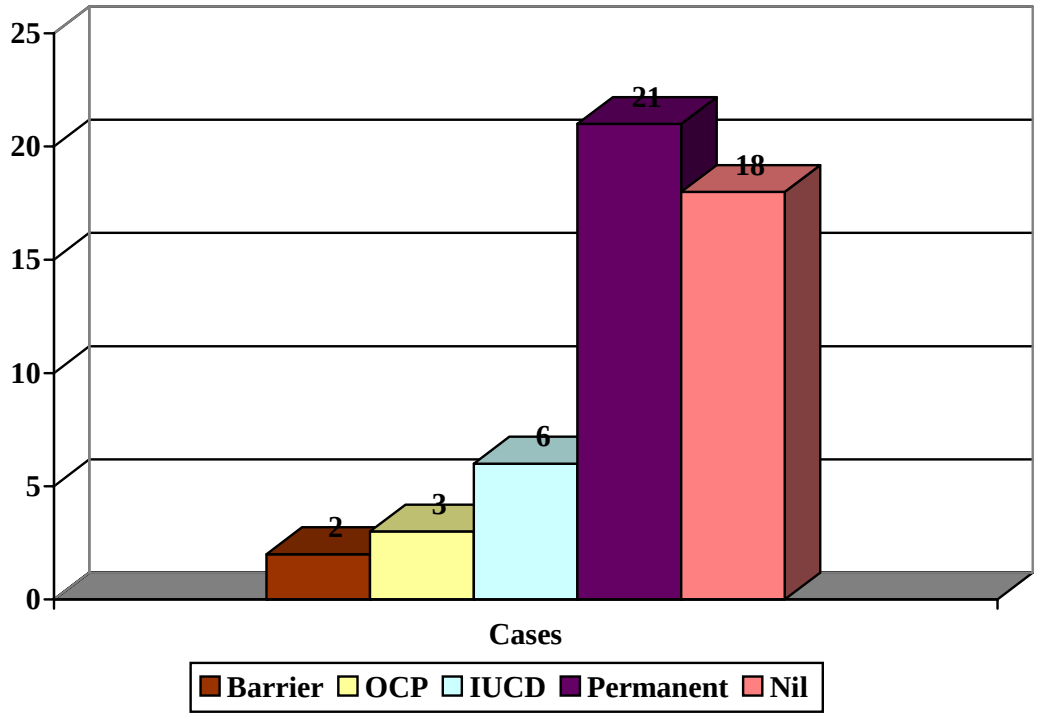
XI. Per vaginal examination

XII. P/R

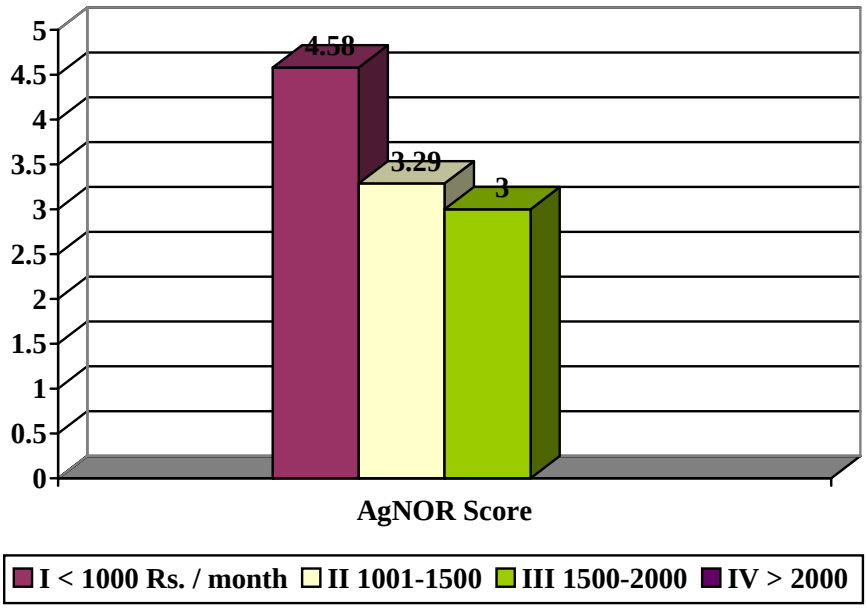
XIII. Biopsy

XIV. AgNOR count

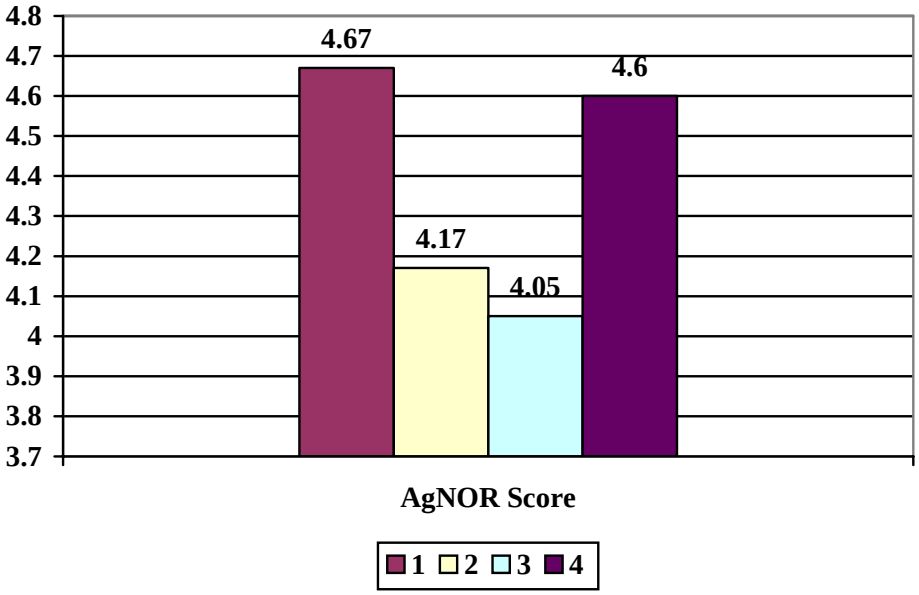
Contraception



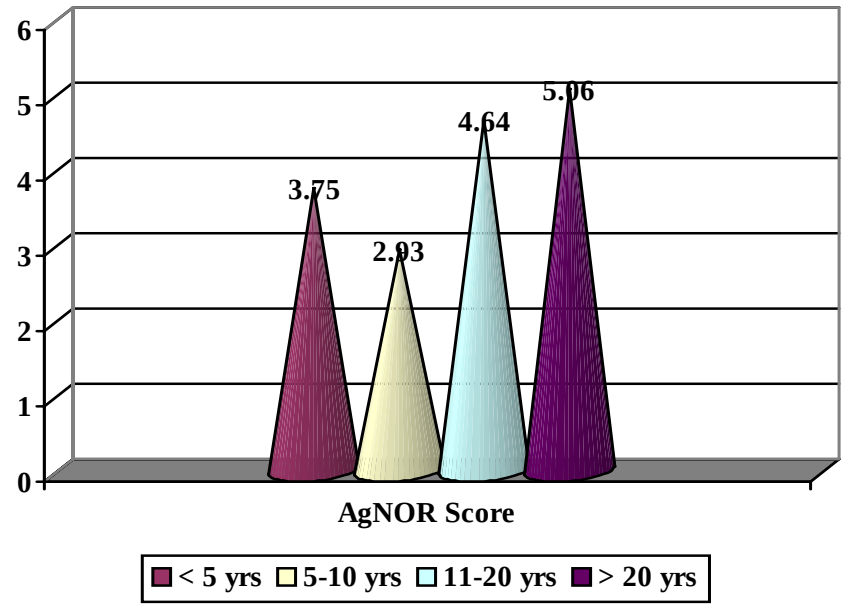
S.E. Status and AgNOR Score



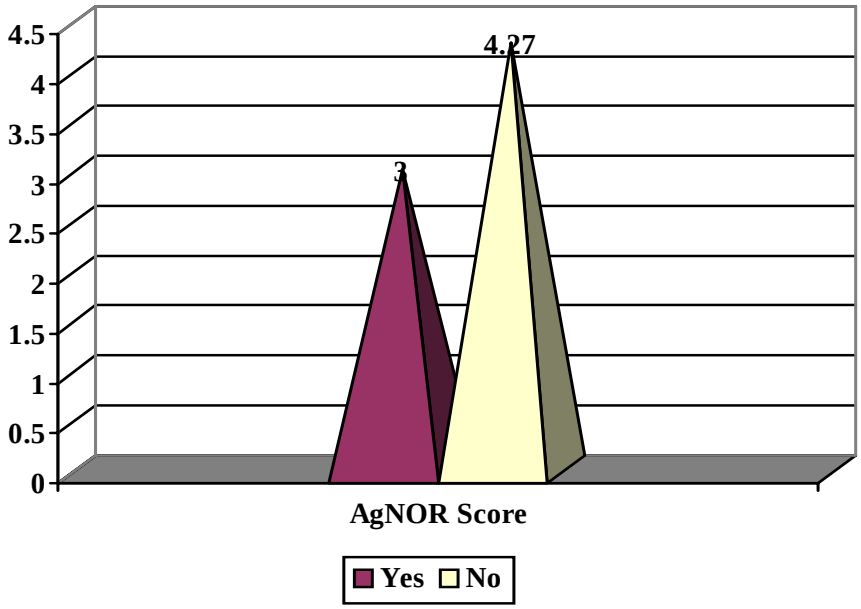
Parity and AgNOR Score



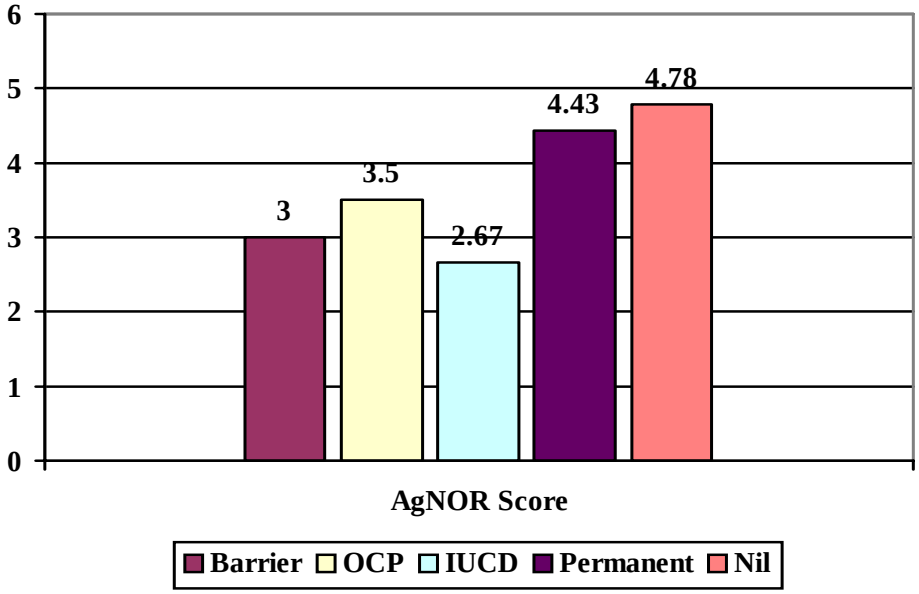
Duration of marital life and AgNOR Score



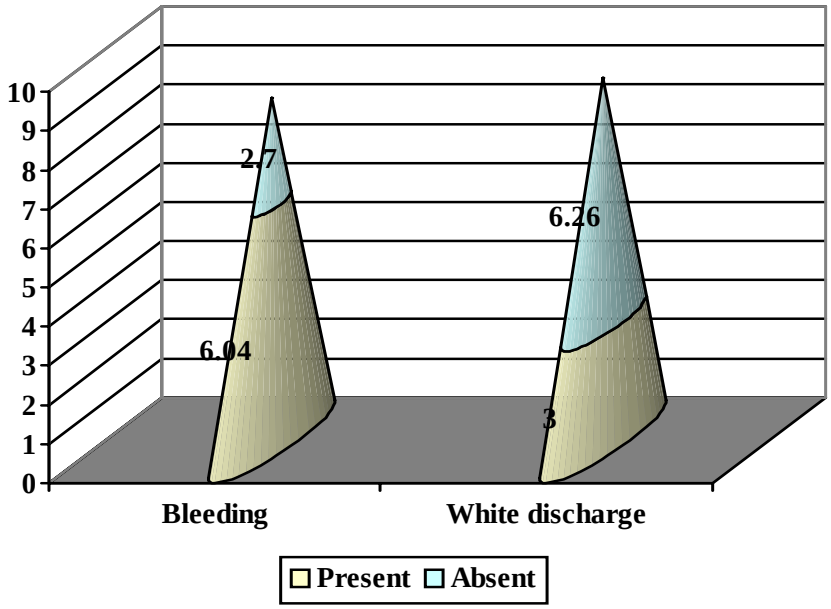
Multiple sex partner and AgNOR Score



Contraception and AgNOR Score



Complaints and AgNOR Score



Clinical appearance of cervix and AgNOR Score

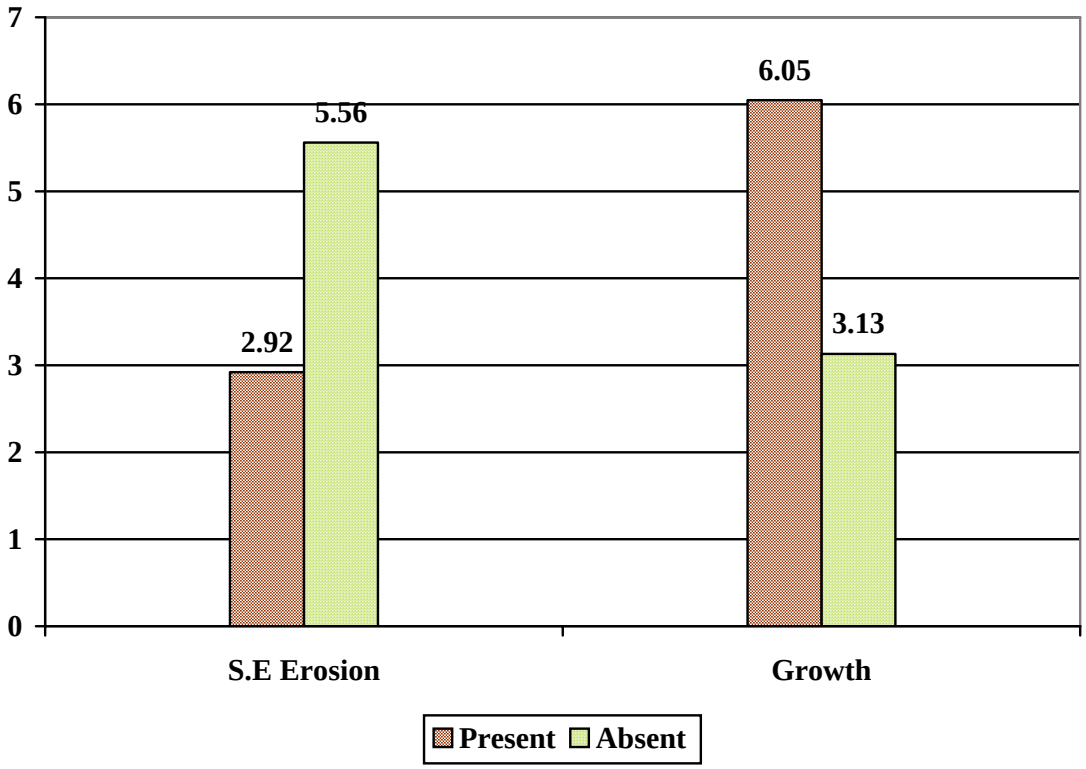


Diagram 1

CELL CYCLE

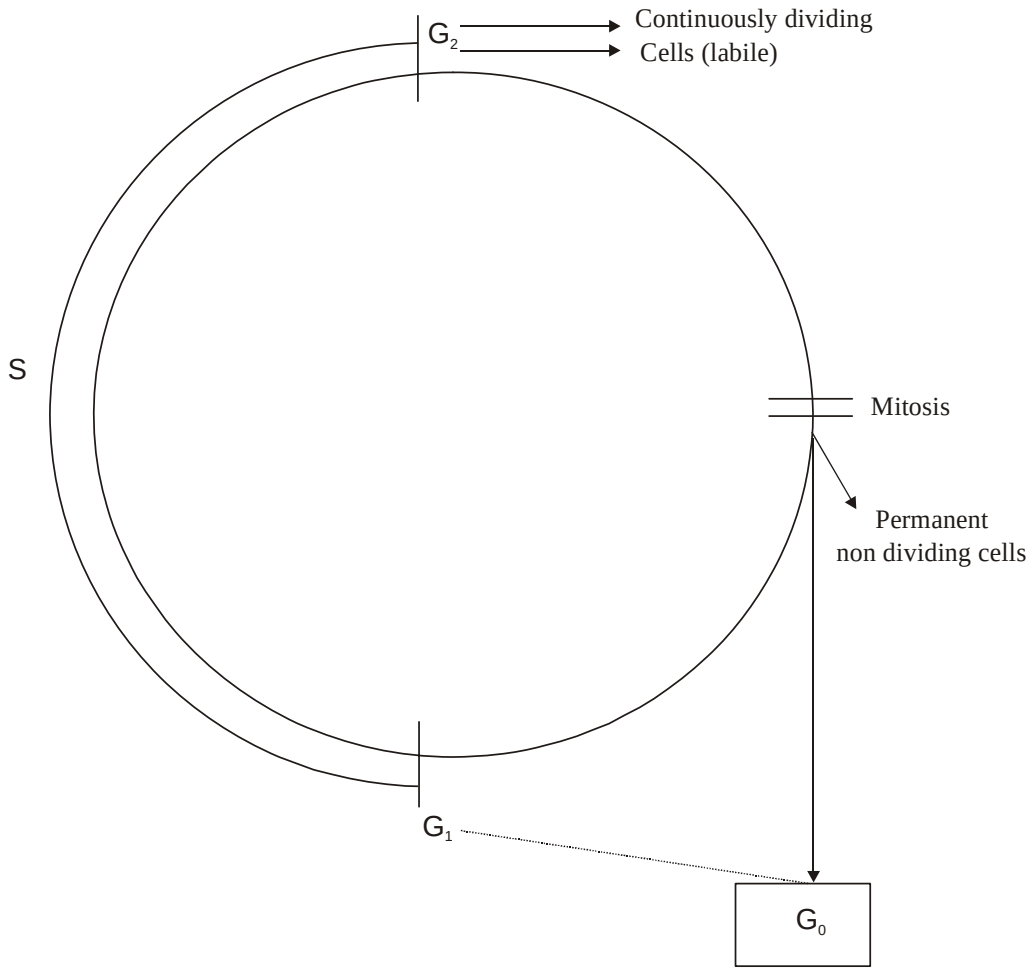


Diagram 2

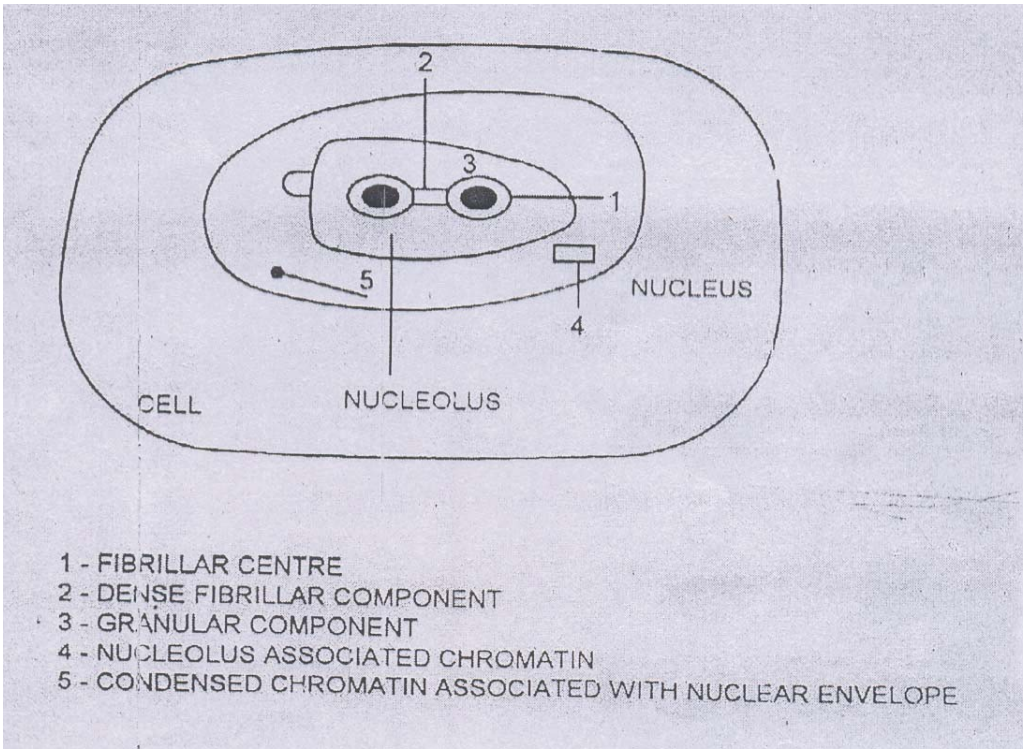

2
3
1
5
NUCLEUS
4
CELL
NUCLEOLUS
1 - FIBRILLAR CENTRE
2 - DENSE FIBRILLAR COMPONENT
3 - GRANULAR COMPONENT
4 - NUCLEOLUS ASSOCIATED CHROMATIN
5 - CONDENSED CHROMATIN ASSOCIATED WITH NUCLEAR ENVELOPE

Diagram 3

TWO RESTING CELLS SHOWING ONE OR TWO NUCLEOLI

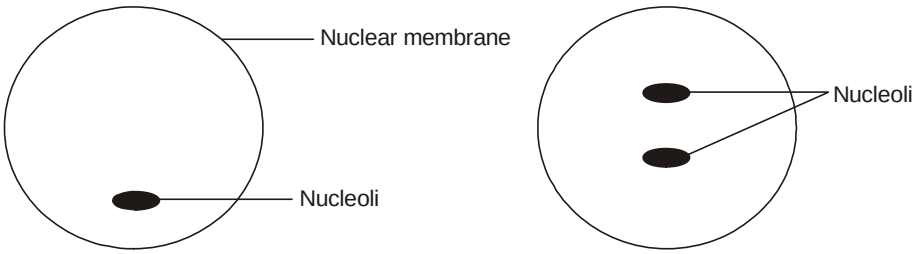


Diagram 4

A TUMOUR CELL SHOWING AgNORs WITHIN THE NUCLEOLI

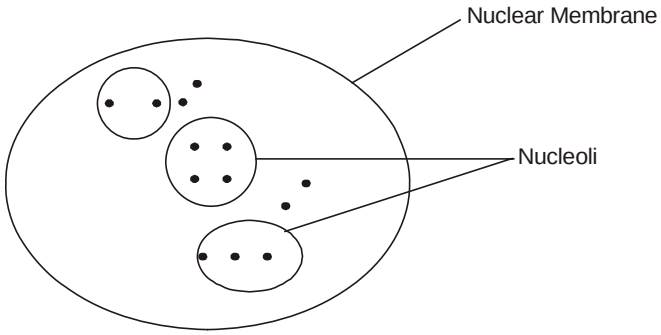
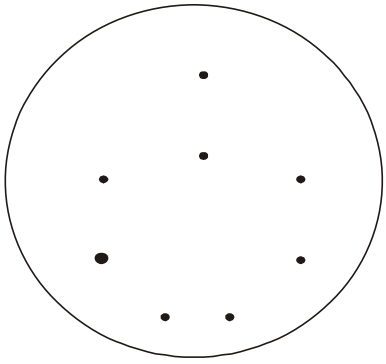
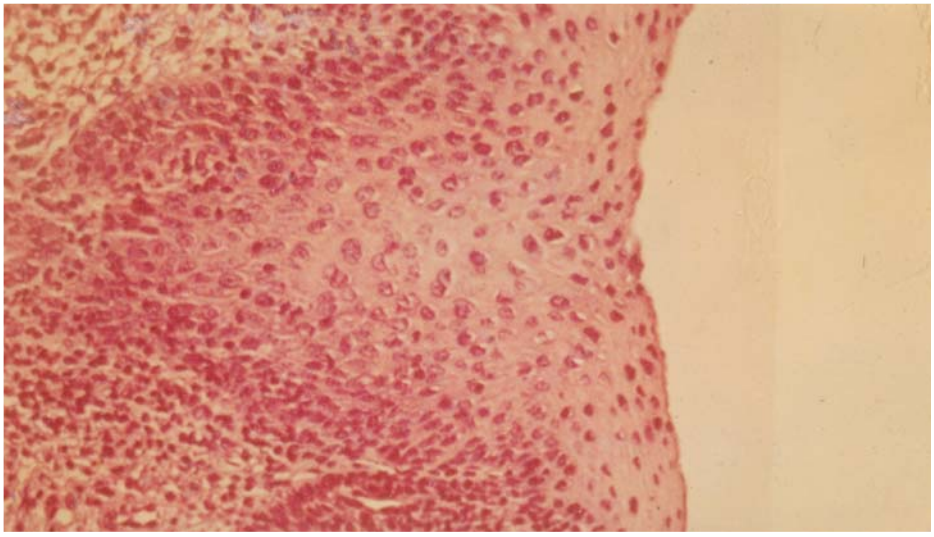


Diagram 5

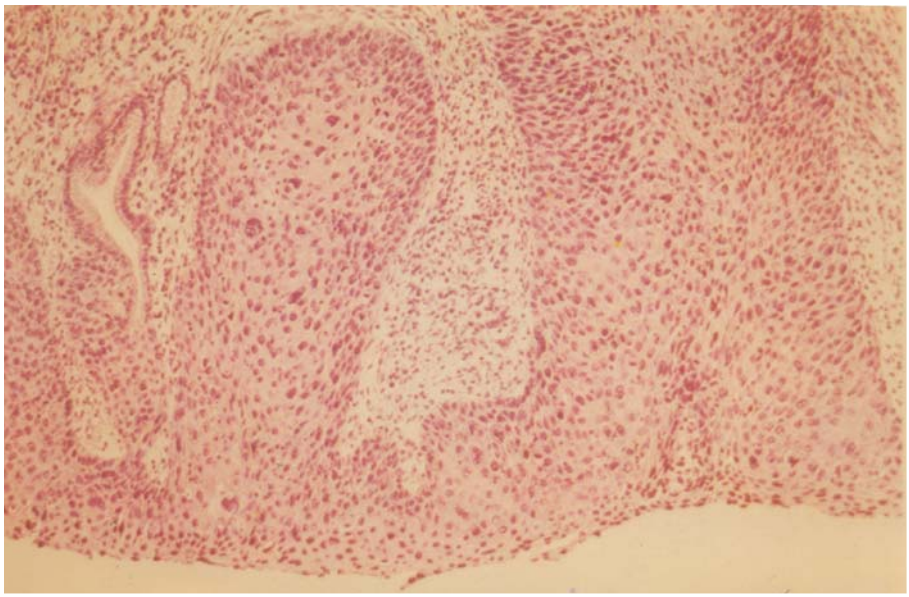
A MALIGNANT CELL SHOWING AgNORs SEEN LYING FREE IN THE NUCLEUS



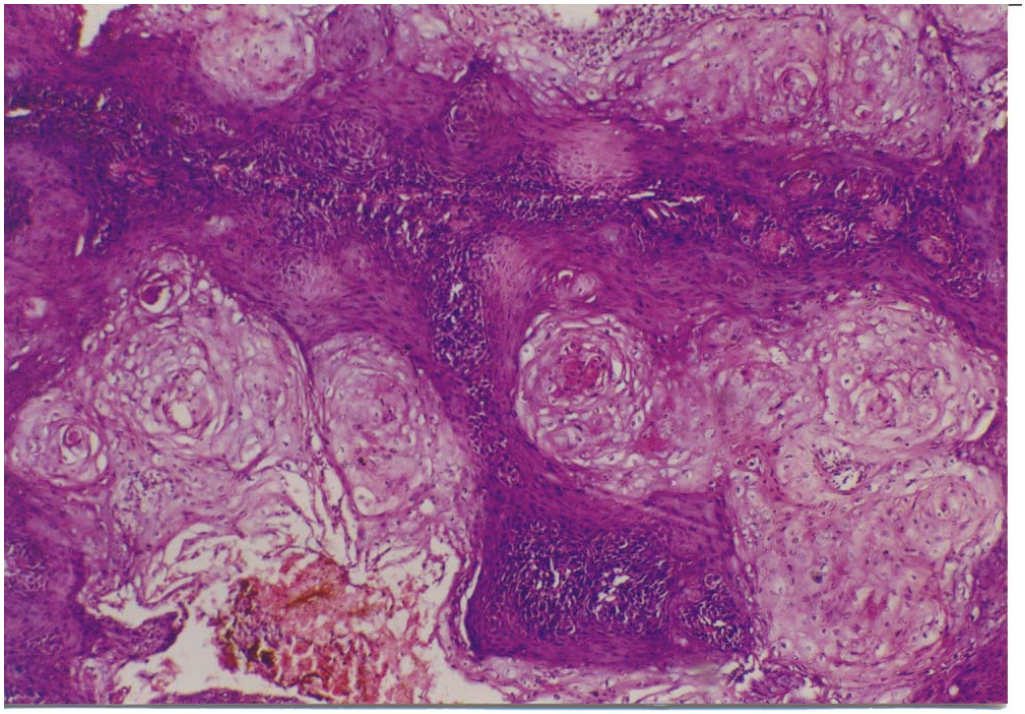
MILD DYSPLASIA



SEVERE DYSPLASIA

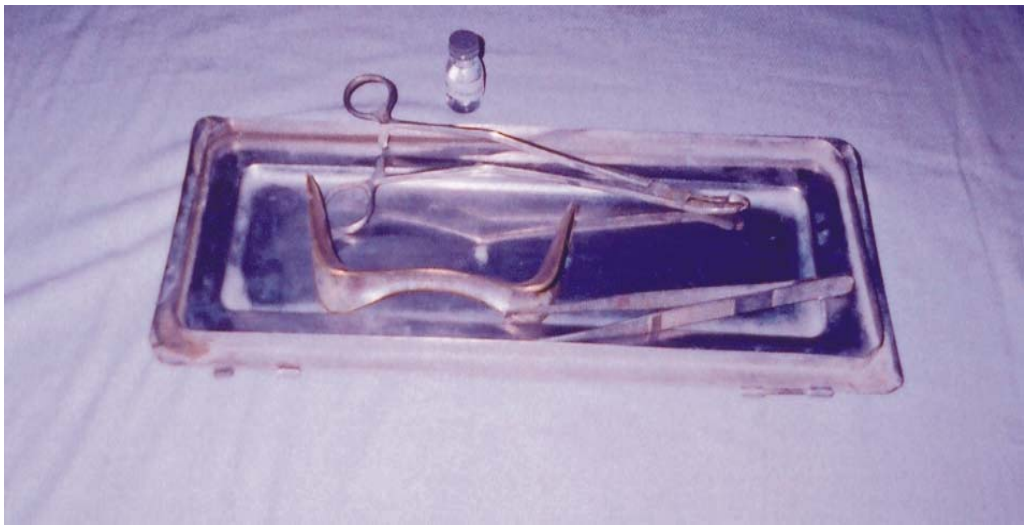


SQUAMOUS CELL CARCINOMA

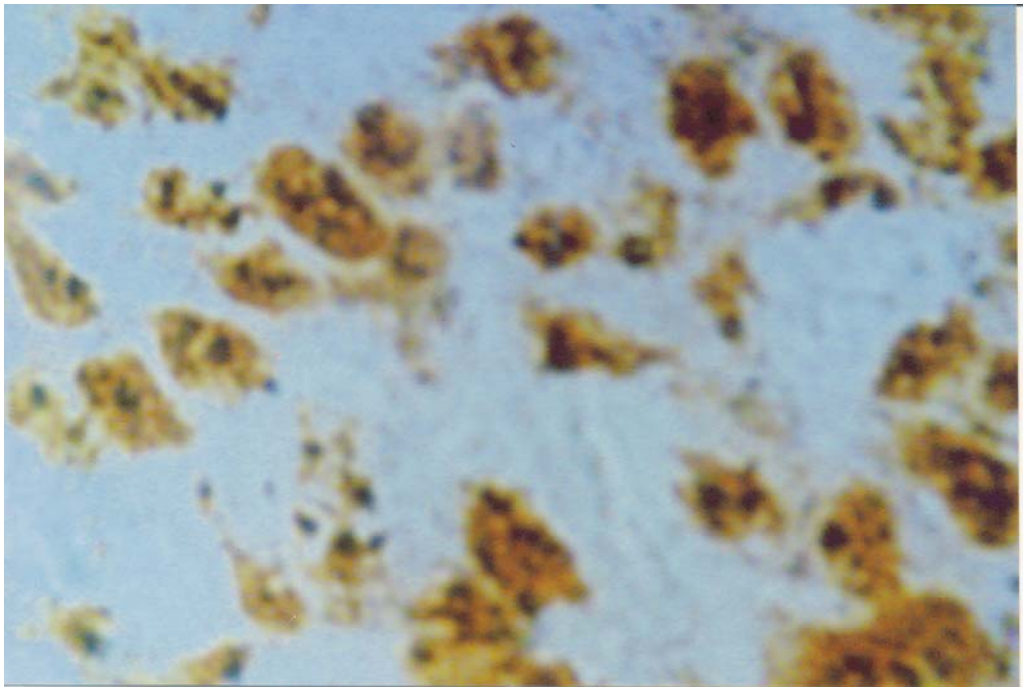


MATERIALS FOR AgNOR STAINING

CERVICAL BIOPSY TRAY



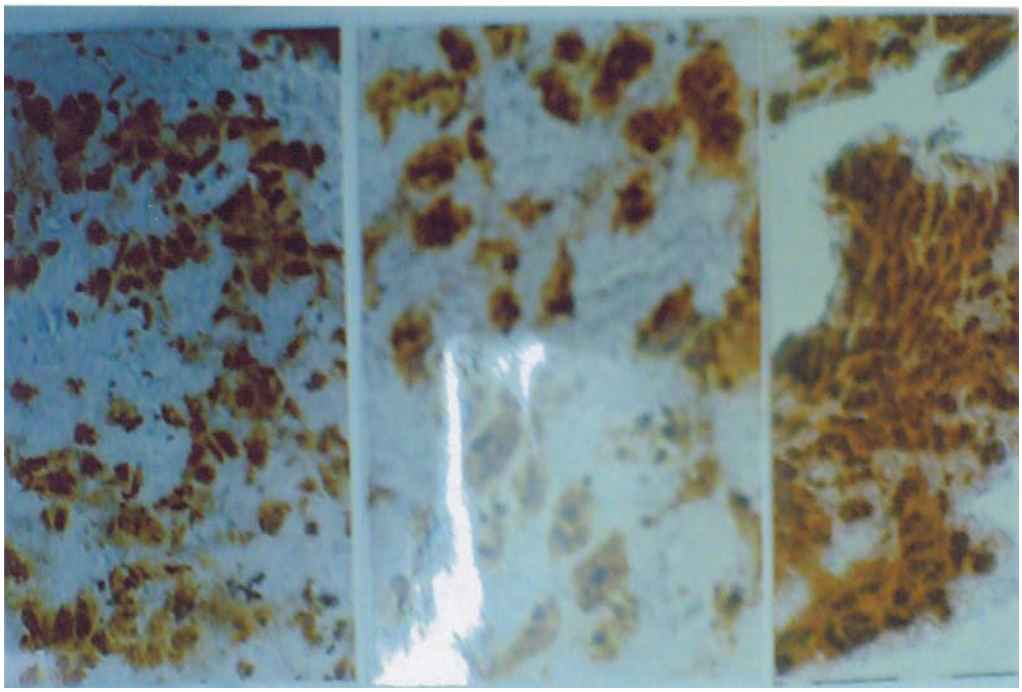
CHRONIC CERVICITIS WITH AgNOR SCORE 3



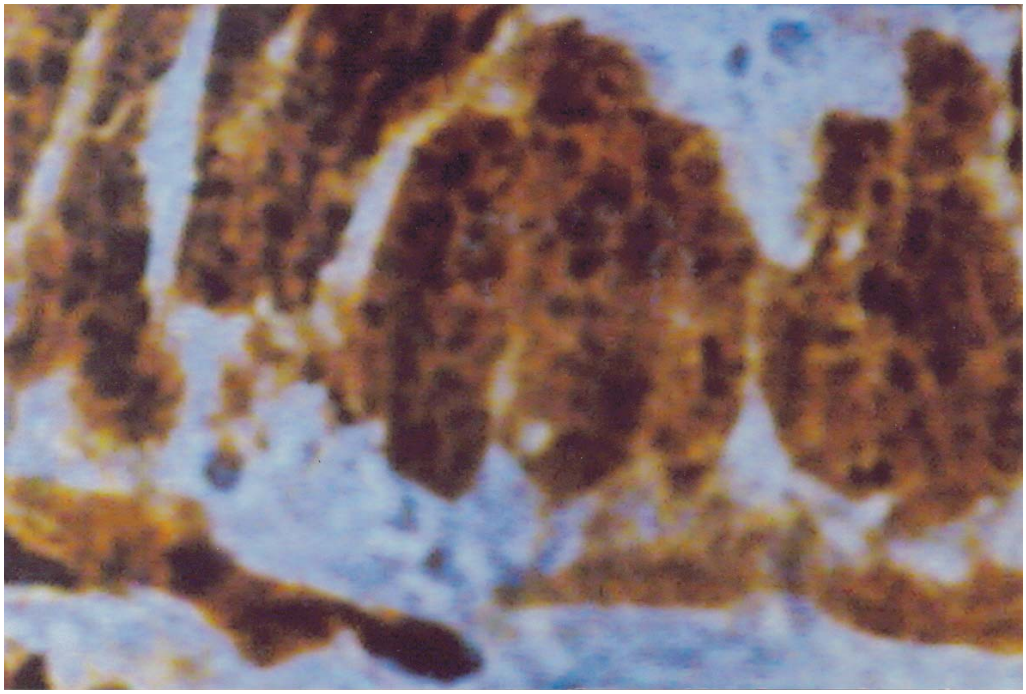
SEVERE DYSPLASIA WITH AgNOR SCORE 4

MILD DYSPLASIA WITH AgNOR SCORE 3

ADENOCARCINOMA WITH AgNOR SCORE 6



SQUAMOUS CELL CARCINOMA WITH AgNORE SCORE 5



MASTER CHART

OP No	Age	Education	S.E.Status (Income per month)	Parity	Duration of m.life(in yrs)	Multiple partners	Contraception	Bleeding	White Discharge	H/o STD	Prev. scr.	Speculum Examination		HPE
												Erosion	Growth	
9054	42	Nil	1001-1500	2	>20	Nil	Perm.	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
7524	28	≤10 std	1001-1500	1	5-10	Nil	IUCD	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
6257	58	Nil	<1000	4	>20	Nil	Nil	PMB	Nil	Yes	Nil	Nil	Yes	SCC mod.diff
7567	60	Nil	<1000	2	11-20	Nil	Nil	PMB	Nil	Nil	Nil	Nil	Yes	SCC mod.diff
0306	38	≤10 std	<1000	3	5-10	Nil	Perm.	Nil	Yes	Nil	Nil	Yes	Nil	Mild dys
7715	54	Nil	<1000	4	>20	Nil	Perm.	PMB	Nil	Nil	Nil	Nil	Yes	SCC mod.diff
7813	60	Nil	<1000	3	>20	Nil	Perm.	PMB	Nil	Nil	Nil	Nil	Yes	SCC mod.diff
5066	45	≤10 std	1001-1500	2	11-20	Nil	Perm.	Nil	Yes	Nil	Nil	Nil	Nil	Ch. cer.
7977	40	≤10 std	<1000	3	11-20	Nil	Perm.	PC	Nil	Nil	Nil	Nil	Nil	SCC mod.diff
5344	60	Nil	<1000	4	11-20	Nil	Nil	PMB	Nil	Nil	Nil	Nil	Yes	SCC mod.diff
145	60	Nil	<1000	1	<5	Nil	Nil	PMB	Nil	Nil	Nil	Nil	Yes	SCC mod.diff
9714	45	≤10 std	1500-2000	2	>20	Nil	Nil	PMB	Nil	Nil	Nil	Nil	Yes	Ade.car
0241	45	Nil	<1000	4	11-20	Nil	Perm.	Nil	Yes	Nil	Nil	Nil	Nil	Ch. cer.
5248	60	Nil	<1000	5	>20	Nil	OCP	PMB	Nil	Yes	Nil	Nil	Nil	SCC mod.diff
8109	40	≤10 std	<1000	3	5-10	Nil	Nil	Nil	Yes	Nil	Nil	Nil	Nil	Ch. cer.
9385	36	Nil	1500-2000	3	11-20	Nil	IUCD	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
2571	45	≤10 std	<1000	3	11-20	Nil	Nil	Nil	Yes	Nil	Nil	Nil	Nil	Ch. cer.
7445	60	Nil	<1000	2	>20	Nil	Nil	PMB	Yes	Nil	Nil	Nil	Yes	SCC mod.diff
9340	40	Nil	<1000	2	5-10	Nil	Perm.	IMB	Nil	Nil	Nil	Nil	Yes	SCC mod.diff
8456	45	≤10 std	<1000	2	>20	Nil	Perm.	IMB	Nil	Nil	Nil	Nil	Yes	SCC mod.diff
5457	30	≤10 std	1001-1500	2	5-10	Nil	OCP	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
9098	35	Nil	<1000	1	>20	Nil	Perm.	PMB	Nil	Yes	Nil	Nil	Yes	Ade.car
9146	32	>10 std	1001-1500	2	<5	Nil	Barrier	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
322	45	≤10 std	<1000	3	11-20	Nil	Nil	IMB	Nil	Nil	Nil	Nil	Yes	SCC mod.diff
832	52	Nil	<1000	2	11-20	Nil	Perm.	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
217	27	>10 std	<1000	3	5-10	Nil	Nil	PC	Yes	Nil	Nil	Nil	Yes	SCC mod.diff
938	50	≤10 std	<1000	4	>20	Nil	Perm.	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
4455	31	≤10 std	1500-2000	2	5-10	Nil	IUCD	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
4346	40	Nil	<1000	3	>20	Yes	Nil	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
867	42	≤10 std	<1000	3	11-20	Nil	Nil	IMB	Nil	Yes	Nil	Nil	Yes	SCC.well.c
5471	41	>10 std	<1000	3	5-10	Nil	Perm.	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
4176	46	Nil	1500-2000	2	>20	Nil	Nil	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.

OP No	Age	Education	S.E.Status (Income per month)	Parity	Duration of m.life(in yrs)	Multiple partners	Contraception	Bleeding	White Discharge	H/o STD	Prev. scr.	Speculum Examination		HPE
												Erosion	Growth	
3857	35	≤10 std	<1000	3	5-10	Nil	Nil	Nil	Yes	Yes	Nil	Yes	Nil	Sev. dys
2689	28	>10 std	<1000	2	<5	Nil	IUCD	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
2896	50	≤10 std	<1000	4	>20	Nil	Perm.	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
5492	48	Nil	<1000	3	11-20	Nil	Perm.	IMB	Nil	Nil	Nil	Nil	Yes	SCC mod.diff
0518	30	≤10 std	<1000	2	11-20	Nil	IUCD	PC	Nil	Nil	Nil	Nil	Yes	SCC Well.diff
6441	36	Nil	<1000	3	5-10	Nil	OCP	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
1055	60	Nil	<1000	4	>20	Nil	Perm.	PMB	Nil	Nil	Nil	Nil	Yes	SCC Well.diff
6868	60	Nil	<1000	2	>20	Nil	Perm.	PMB	Nil	Nil	Nil	Nil	Yes	SCC Well.diff
3926	40	Nil	1500-2000	2	5-10	Nil	Perm.	Nil	Yes	Nil	Nil	Yes	Nil	Ch. cer.
4190	24	>10 std	1001-1500	2	5-10	Nil	IUCD	Nil	Yes	2	2	Nil	Yes	Mild dys
4579	30	>10 std	<1000	3	5-10	Nil	Barrier	Nil	Yes	2	2	Yes	Nil	Metapla
4711	35	Nil	<1000	3	11-20	Nil	Perm.	PC	Nil	2	2	Yes	Nil	Sev. dys
3141	35	≤10 std	<1000	3	5-10	Nil	Nil	Nil	Yes	2	2	Yes	Nil	Mild dys
4675	27	>10 std	1001-1500	2	<5	Nil	Nil	Nil	Yes	2	2	Yes	Nil	Sev. dys
8254	39	Nil	<1000	3	>20	Nil	Perm.	Nil	Yes	2	2	Yes	Nil	Mild dys
2652	53	Nil	<1000	4	>20	Nil	Nil	Nil	Yes	2	2	Yes	Nil	Ch. cer.
2611	40	Nil	<1000	3	11-20	Nil	Nil	Nil	Yes	2	2	Yes	Nil	Ch. cer.
5803	60	Nil	<1000	4	>20	Nil	Perm.	PMB	Yes	2	2	Yes	Nil	Sev. dys

ABBREVIATIONS

S.E. Status - Socio Economic Status

OCP - Oral contraceptive pill

IUCD - Intra uterine contraceptive device

Perm. – Permanent

PMB – Post menopausal Bleeding

PC – Post coital bleeding

IMB – Inter menstrual bleeding

Mild dys. – Mild dysplasia

Sev. dys. – Severe dysplasia

Meta pla. – Metaplasia

Ch. Cer. – Chronic cervicitis

Ade.car. – Adeno carcinoma

SCC Well diff. – Squamous cell carcinoma well differentiated

SCC Mod. diff. – Squamous cell carcinoma moderately differentiated